

Pipeline Blockage Cleared by House of Representatives

UNDER the pressure of intense lobbying from the oil industry and from the Administration, members of the US House of Representatives last week followed the lead of their colleagues in the Senate by passing a reckless piece of legislation. By a vote of 356 to 60, the House cleared all legal challenges to construction of the trans-Alaska oil pipeline and brushed aside the suggestion that a thorough study should be conducted of an alternative route across Canada to the US Midwest. By passing the bill the House attempted to usurp the power of the courts by deciding for them that the Department of Interior has fulfilled the mandate of the National Environmental Policy Act (NEPA) in granting a right of way for the pipeline. It is the latter provision which is not only unwise but also unnecessary, and which threatens to undermine one of the most important environmental laws in the United States.

The trans-Alaska pipeline, a proposal to transport crude oil from Alaska's North Slope to the ice-free port of Valdez, has been snarled in legal tangles for more than three years. It may thus seem contradictory to refer to Congress's actions as reckless, but members of Congress were stampeded into passing a highly questionable piece of legislation by threats of the energy crisis.

In short, both the House of Representatives and the Senate have now passed bills which clear away a particular legal technicality which was barring the way to construction of the pipeline. But, instead of stopping there and allowing the courts to decide other outstanding legal issues, supporters of the pipeline also added a provision to the bills which attempts to decide those other legal issues for the courts.

Environmental groups have, during the past three years, fought a long court battle against construction of the pipeline, arguing that the Secretary of the Interior has not fulfilled all the dictates of NEPA. The act requires all departments and agencies of the federal government to produce environmental impact statements giving details of the effects on the environment of proposed projects, and analysing alternatives to the proposed course of action. Opponents of the trans-Alaska pipeline have argued in the courts that the Secretary of the Interior has failed to meet the obligations of NEPA, chiefly because insufficient attention has been paid to an alternative route through Canada. They lost their case in a federal court, but appealed the ruling, and in February this year an appeals court astonished everybody by revealing that the Minerals Leasing Act of 1920 forbids the granting of a right of way wide enough for construction of the pipeline. At that time the appeals court decided not to rule on the environmental questions until the Mineral Leasing Act had been modified, and that was the chief purpose of the legislation passed last week.

The provision exempting the pipeline from further court review under NEPA was inserted into the Senate bill in the form of an amendment offered by the two Senators from Alaska, Ted Stevens and Mike Gravel. The amendment was finally agreed to by the Senate by a vote of 49 to 48, after it had been vigorously opposed by

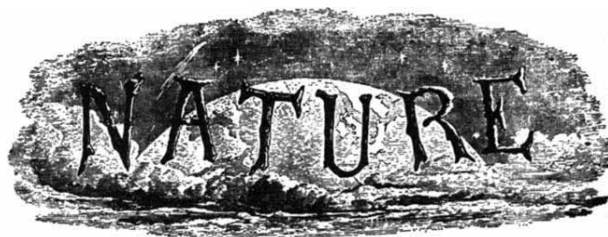
Senator Henry M. Jackson, one of the chief supporters of the trans-Alaska pipeline. Similarly, it was inserted into the House bill by a majority of one in the Interior Committee, and survived a test on the House floor by 221 votes to 198.

NEPA has been used by environmentalists to oppose a variety of government projects, and it has been responsible for a number of significant improvements in the design of projects and in the development of government policies. The act, for example, provided the level for forcing the Atomic Energy Commission to take into account environmental factors other than radiation in the licensing of power plants, and it is also likely ultimately to force cancellation of the extremely dubious attempts to free trapped natural gas with nuclear explosives. Moreover, the court suit filed under NEPA against the trans-Alaska pipeline has unquestionably forced the oil companies to adopt a much more acceptable design for the venture.

Actions which may lead to weakening NEPA are thus not to be taken lightly, but in its cavalier approach to the legal obstacles lying in the path of the pipeline, Congress has set an unfortunate precedent. Can it not be argued, for example, that because the breeder reactor will be so vital to providing energy in the 1990s, that it, too, should be exempted from NEPA review?

Equally important, in trying to deny further environmental review of the pipeline, Congress has cut off consideration of an alternative route through Canada. There are, clearly, many drawbacks to the Canadian route, including the ambivalent attitude of the Canadian government, but as it is almost certain that a gas pipeline will be built from the North Slope through Canada to the US midwest, there is a case for studying the impact of running an oil and gas line side by side. Congress, however, was persuaded by skilful lobbying from the oil industry, that the energy crisis demands not only that Alaskan oil should reach the markets without delay, but that the need is great enough to call for unwise action.

100 Years Ago



A GREAT earthquake occurred at Valparaíso early on the morning of July 8. There were six shocks in succession. Many families took refuge in the streets, the damage to private houses as well as to the public buildings being considerable; and many deaths were reported. A statue lately erected to Lord Cochrane was wheeled half round on its pedestal. The earthquake was observed to come from the east, and was felt as far south as Curico.

From *Nature* 8, 314, August 14, 1873.



OLD WORLD

European Space Out of the Wood?

LAST week's meeting in Brussels of the European aerospace ministers, in spite of its long hours and histrionic delays, could prove a major advance in European space history. But the ministers and their civil servants are not yet out of the woods.

The agreement that finally emerged provides for Mr Michael Heseltine's European Space Agency to come into existence on April 1 next year (three months later than originally planned) and sets out—at last—which projects are going to be supported and by whom. Spacelab, L-3S (the French launcher), and MAROTS, the maritime satellite that the European Space Research Organization has been studying, are the projects that have been given the European blessing, and in the end all the nations agreed to contribute to all three projects—the only reservations being that Italy, Denmark, Sweden and Norway have still to decide on the amount of their contributions.

Feeling in the European aerospace industry is chiefly one of relief that a clear programme is finally emerging. It has been said, however, that in working to get the new agency set up, Mr Michael Heseltine, Britain's Minister for Aerospace and Shipping, has given too much away in terms of real projects. The British proposal to build a Geostationary Technology Satellite as a prototype maritime satellite was dropped in favour of MAROTS (see *Nature*, 243, 181; 1973), and Britain has even agreed to contribute a largely nominal sum (2.6%) to the French launcher programme. The feeling in Whitehall, however, is that the establishment of the agency is the key point, and any contribution to the L-3S is less an important change of principle than a minor concession made to ensure that the agency gets off the ground. When the project gets under way Britain will not be a formal partner to the programme. Britain's attitude through the last few ministerial meetings has been that it is more important to plan for a rational future than to win short term victories over particular projects that may damage the long term interests of a concerted European space effort.

British expenditure in space in fact is going to be small over the next few years—a mere £30 million or so—and a large share of that—about £17 million—will be spent on MAROTS as Britain is to pick up 56% of that bill.

The problems left to be resolved are the extent of the Italian and Scandinavian contributions, the precise machinery under which the new agency will

operate, and—most importantly—the way in which new projects, which in the old days would have been national projects, are to be integrated into the new organization.

The agreement for Europe to go ahead with the £130 million Spacelab project will now be signed on August 14, although there will be a reservation written in that the agreement is void if the countries that have still to decide their contributions to the overall package fail to provide the necessary funds by September 18, the deadline that was set for them at Brussels. There is, however, surprising confidence in European space circles that the money will be found. Considerable pressure will be put on the Italians in particular not to destroy an agreement that has taken months to negotiate. Even if there is a

shortfall, it is felt that the countries that are already committed will increase their contributions.

The precise machinery of the new agency has still to be worked out, but it is already clearly understood that it will have a strong central management on the lines of ESRO rather than on the lines that helped lead ELDO to ruin.

Britain's chief aim in all this will be to see that all new projects dreamt up in the future will be offered to the new agency. This is one of the reasons why the national GTS programme was dropped in favour of the admittedly British dominated MAROTS programme. Another reason is that if money is to be made out of applications satellites they need to be run on a multinational basis.

SCIENCE POLICY

Dahrendorf's Solution

PROFESSOR Ralf Dahrendorf, European Commissioner for Scientific Research and Education, has produced his plans for coordinating the science and technology policies of the nine community members. Headstone of the six point plan is a new committee for Scientific and Technical Research (CREST) to add to the other acronyms that already litter the European research scene.

If approved by the council of ministers at their October meeting this committee—whose recommendations in its early years at least will not be binding—will have the task of coordinating national policies by pinpointing the areas into which each country should put more, or less, effort. The precise form the committee will take is still under discussion. If the ministers do accept the proposals—and given the fate that met Commissioner Spinelli's proposals (see *Nature*, 243, 128; 1973) this can by no means be certain—it will mean that Europe finally will have a common research policy.

The other chief points in the plan allow for the commission to take a share in the proposed European Science Foundation that is currently being put together by the academies and research councils of Europe. Although no sum has been mentioned it is possible that the commission will put a small amount of money into the new body each year—perhaps 200,000 units of account (£80,000)—in order to gain the right to be involved. Professor Dahrendorf has

also highlighted a series of projects that he is asking the council of ministers to approve. These include studies on medical and social research, energy policy—including direct conversion, the transport of energy and environmental and regional energy policies—as well as an industrial policy that includes work on materials and computers.

Where this work is to be done has not yet been decided, but possibly contracts would be placed in industry, national research institutes and the community's Joint Research Centre.

The programme includes some old favourites of proposed European cooperation such as a community-wide scientific information network and the setting up of a European bureau of standards based around the existing Bureau of Nuclear Standards at Geel in Belgium. The new bureau, however, would have far wider interests, covering for example standards in pharmaceuticals and medicine, in food conservation, pesticides and telephone and telecommunications links.

The final point in the proposal is that the commission should join the futures business by setting up a group to examine likely developments in the next thirty years along the lines of the MIT and Club of Rome studies. The group has been dubbed "Europe+30".

If the council of ministers accepts the proposals it is likely that the PREST group that advises the council of ministers will be dissolved, but the recently set up CERD group, whose purpose is to advise the commission on the policies it should adopt, will continue in existence.

BUSINESS

Nuclear Trading

A PLEASANT past and a promising future characterizes the annual reports of British Nuclear Fuels and The Radiochemical Centre, published last week.

These two off-shoots of the United Kingdom Atomic Energy Authority have just celebrated their second year as independent entities, and both are now operating at a healthy profit. But British Nuclear Fuels report is noteworthy chiefly for the news that the government is to advance the greater part of the capital required to build the gas centrifuge uranium enrichment plant that is currently planned for completion in 1976. Under a tripartite agreement between Britain, West Germany and the Netherlands two demonstration plants designed to produce 200 tonnes a year are being built at Capenhurst and Almelo to be run by Urenco and Centec, the two companies set up under the agreement to construct the plants. If all goes well enrichment capacity will be increased to 2,000 tonnes a year by 1980.

As Sir John Hill, BNFL's chairman, points out in the report, the capital sum—which is undisclosed but is thought to total between £50 and £60 million—is substantial in relation of BNFL's earnings, which last year rose £1 million to £65 million. In spite of the three way sharing of construction costs the sum is still large; under the terms of the guarantee the government will be repaid out of the profits from the undertaking.

The report also states that BNFL will not be taking part in the second stage of the French Eurodif studies of diffusion plant. France is eager to build a large diffusion plant, but the economics of the operation are such that only one large plant that is run at almost full load can be built. BNFL took part in the first stage of the international studies of such a plant but declare that "the first year's work . . . confirmed the company in its view that the centrifuge process was likely to offer better prospects of producing enrichment for the European and world market at competitive prices in the medium and long term. The company is not taking part in the second year of the study".

The report also shows that BNFL's profits remained static at £10 million although the percentage profit rose by 1.7% to 15.8%. Sales only increased £1.6 million, chiefly due to the lack of new orders for nuclear power stations and the fact that the Advanced Gas Cooled Reactors are not yet on load. BNFL managed to sell some reload fuel for light water reactors to the Netherlands, "but a major expansion in this field is unlikely unless water reactors are introduced into the UK power programme", according to Sir John Hill.

The Radiochemical Centre for its part increased its sales by over 25% to £7.6 million. The profit before tax rose 61% to £1.3 million, representing a return on capital after loan interest charges of 28%. Seventy per cent of the centre's business is overseas, the largest growth area being radiopharmaceuticals.

Considerable investment in new facilities is planned over the next few years, and the centre's chairman, Sir Charles Cunningham, states that the company's staff structure—essentially that inherited from the United Kingdom Atomic Energy Authority—is to be revamped "to meet the needs of a commercial company operating in world markets".

ECOLOGY

Detecting the Changes

from a Correspondent

How is environmental change (brought about by man's activities or by natural causes) to be assessed? This broad question received a detailed examination in London recently when the Natural Environment Research Council held a one day seminar under the title the detection and assessment of significant biological change.

The meeting's conclusion seemed chiefly to be that there is a great need for more background data, more scientific effort and, of course, more resources. But on the way to that conclusion some interesting points were raised.

Mr R. J. H. Beverton, secretary of NERC, highlighted the problem by pointing out that one of the chief difficulties lies in the diversity of the living world and in its, mostly unrecorded, variability. As many man-made influences are enhancements of natural ones it follows that their effects are difficult to detect and quantify. It is often difficult, Mr Beverton said, to distinguish between the signal and the background noise.

Many monitoring programmes are already under way whose objective is to detect adverse trends. The methods employed are almost always physical or chemical—for instance measuring temperature, salinity, dissolved oxygen or suspended solids. The question is how important are the changes to biological communities, and how can these fluctuations be detected before damage occurs?

Dr J. W. G. Lund of the Freshwater Biological Association declared that while it is often possible, with hindsight, to explain changes that have already taken place in the freshwater environment, it is quite a different matter to predict them. Often, he said, we do not even know what should be observed. This view was supported by Dr J. M. Hellawell who, as a NERC research fellow, has examined several

schemes for biological monitoring of rivers. He concluded that the schemes he studied were often inaccurate and sometimes misleading when taken on their own.

Predicting changes on land seems little easier. Schemes for aphid and moth monitoring that have been run on a national scale for a number of years were described by Dr L. R. Taylor of the Rothamsted research station. Again, changes are easily detected in the populations, but it is so far impossible to distinguish between short and long term climatic changes, alterations in land use or other man-made influences.

In the marine environment changes cannot yet be predicted accurately, while on the shoreline too little is known about the biological interactions in shore communities to allow monitoring to take place.

Other monitoring programmes run into further difficulties over the subjective element introduced by the observers who provide the data—for example the monitoring of bird populations by the British Trust for Ornithology and the mapping of plant species from Monks Wood research station.

For monitoring to be effective it may, in the end, have to concentrate on a limited closely defined objective. Nonetheless it is still hoped that some schemes can be conceived to provide an overall index of environmental quality. In response to this possibility NERC has just appointed two working groups to examine biological surveillance in the marine and terrestrial environments.

SPACE

Mars 6 Stirs Speculation

from our Cosmology Correspondent

THE launch of Mars 6 on August 6, just one day before the recent "window" for Mars launches closed, makes the present series of probes the first Soviet triple venture to the red planet. Together with the wording of official communiqués about the launch, this has encouraged renewed speculation that Mars 6 might be the awaited soft lander, possibly carrying a "Marsokhod".

While Mars 4 and Mars 5 are described as being "analogous in structure and purpose" (see *Nature*, 244, 249; 1973), Mars 6 is different in design from the two probes now three weeks ahead of it on the way to Mars, according to TASS. The official releases say that Mars 6 will use Mars 4 in carrying out its exploration, a choice of phrase which has been taken as hinting strongly that Mars 6 will carry out a landing on the planet and use Mars 4 to relay messages back to Earth. In that case, Mars 5 might well incorporate a backup facility for Mars 4.

NEW WORLD

Advice of NIH Officials Ignored

by our Washington Correspondent

A SERIES of memoranda which surfaced last week on Capitol Hill provide a fascinating glimpse of executive infighting which preceded the recent cutbacks in funding for biomedical research. Written late last year by Dr Robert Q. Marston, then Director of the National Institutes of Health, and Dr Frank J. Rauscher, Jun., Director of the National Cancer Institute, the memoranda show that the advice of top NIH officials was completely ignored by the Office of Management and Budget, and they also bear witness to the strong, independent stand taken by Dr Marston before he was fired as NIH director in January.

The memoranda were made public by Senator Mike Mansfield, the Senate Majority Leader, and Senator Warren G. Magnuson, chairman of the appropriations subcommittee which deals with the NIH budget. In releasing them, the two senators are clearly marking out the battle lines for yet another struggle between Congress and the Administration over the size of the budget for the Department of Health, Education and Welfare—four times in the past four years Congress has passed HEW appropriations bills which President Nixon has vetoed because he considered them to be inflationary, and it is likely that the same thing will happen again this year.

Late last year, when it became clear that federal expenditure was greatly outstripping revenues, President Nixon instructed the OMB to cut back on expenditures in order to head off a tax increase. The National Institutes of Health were particularly hard hit by the squeeze, for the OMB proposed to apply the knife not only to planned expenditures in the 1974 budget, which was then in the final stages of preparation, but also to 1973 expenditures. The proposal was to reduce the budgets of all the institutes except for the National Cancer Institute (NCI) and the National Heart and Lung Institute (NHLI), both of which were too politically sensitive to cut, and to eliminate entirely the NIH training and fellowship programmes.

When that proposal was put to Dr Marston, he fired off a memorandum to the Secretary of Health, Education and Welfare deploring the cuts and suggesting that "the President should be made aware of the long-range impact that this will have to the health of the Nation". The memorandum, dated December 19, pointed out that Marston had previously expressed concern that increases in the budgets of NCI and

NHLI were being made at the expense of other institutes, and added that the final OMB proposals "would mean a program over a two-year period from fiscal year 1972 to fiscal year 1974 where cancer increases by \$127 million, heart increases by \$23 million and the rest of biomedical research *decreases* by \$111 million — this, in spite of assurances that the highly political and visible programs would not grow at the expense of basic programs". As for training programmes, Marston acknowledged in the memorandum that they had been axed by OMB in spite of opposition from NIH officials and from the Secretary of HEW.

In spite of the fact that the National Cancer Institute escaped from the budgetary squeeze with an increase of funds, however, it is clear from two memoranda written by Dr Rauscher that the institute suffered badly from OMB's knife. NCI officials originally put in a budget request for the 1974 fiscal year of \$640 million, with the objective of increasing the cancer research budget to \$1,000 million over the next few years. Clearly, the \$640 million was an opening bid in the negotiations between NCI and OMB, and NCI officials knew that they would not get everything they wanted. But they were not expecting the request to be trimmed to \$500 million, which eventually happened.

Asked in November to highlight the consequences if NCI's budget request were cut from \$640 million to \$456 or \$550 million, Rauscher said in a memorandum to OMB that "a signifi-

cant decrease from the \$640 million, which we have requested for 1974, will seriously limit NCI's ability to carry out the objectives the Executive and members of Congress have so often enunciated". Among the consequences of a budget of \$550 million that Rauscher lists in the memorandum are restriction of the "expansion of immunologic treatment of cancer into clinical trials", at least a year's delay in letting industrial contracts to produce automated screening of Pap tests, "instead of introducing 8 to 10 new antitumour agents into general medical practice, at best we could introduce only 5 or 6", efforts to develop less hazardous cigarettes would have to be cut back or terminated, plans to expand cancer research at Fort Detrick "cannot be implemented", and "a deep cut would be necessary in the construction program in order to preclude major reductions in the ongoing program of competing renewal and new grants". In the event, however, OMB ignored Rauscher's plea and cut the NCI budget request to \$500 million.

Congress has not yet completed action on the 1974 budget for the Department of Health, Education and Welfare, but it is already clear that it will greatly increase the Administration's request. The House of Representatives, for example, has passed an appropriations bill that is some \$1,800 million greater than President Nixon requested, and to judge by the comments made by Magnuson last week, the Senate is likely to follow suite. Clearly, the HEW budget is again destined to become a political football.

FUNDING

No Money Yet

by our Washington Correspondent
CONGRESS adjourned last week without completing action on any major appropriations bills affecting science and technology. But at least NASA and the National Science Foundation can be reasonably certain of how much money Congress is willing to give them, for the Appropriations Bill, allotting funds to those two agencies, has now been passed by the House of Representatives and will probably be taken up by the Senate early in September. There are still some minor disagreements to be sorted out on the Senate floor, but NASA is likely to end up with some \$3,002 million—\$13.9 million less than the Administration requested—and the National Science Foundation is likely

to get \$569.6 million—\$13 million less than the administration requested.

Those figures were contained in a conference report which settled differences in the Appropriations Bills passed earlier this year by the House of Representatives and the Senate. The House passed the conference report shortly before the adjournment, but the Senate did not act on it because it contains two controversial provisions, unrelated to the two science agencies, which require some debate. At least astronomers can be thankful for one thing—the conference report includes \$5 million for the Very Large Array Telescope System. Although that figure is only half of the original budget request, the House of Representatives had originally cut out all the money for the VLA and restoration of half the request will at least allow construction to start this year.

AIR POLLUTION

Detroit Blues

by our Washington Correspondent

AUTOMOBILE manufacturers last week wrung another concession out of the Environmental Protection Agency. But, to judge by the volume of complaints from Detroit, the EPA's concession fell well short of capitulation. Mr Robert Fri, acting EPA Administrator, announced that he has granted the manufacturers their request for an extra year to meet stringent limits on the amount of oxides of nitrogen in car exhausts, but he also set interim limits which the manufacturers insist are still too tough.

The decision parallels an earlier, controversial decision taken by Mr Ruckelshaus when he was administrator of EPA, to allow the manufacturers an extra year to meet emissions standards for hydrocarbons and carbon monoxide (see *Nature*, 242, 491; 1973). The Clean Air Act specifies that all 1976 model cars sold in the US must emit no more than 0.4 g of oxides of nitrogen per mile—about 90 per cent less than 1971 models—but it also allows the EPA Administrator to delay imposing the standards by a year if he finds that it cannot be met on time. That, essentially, is what Mr Fri decided last week, but he also imposed a limit of 2 g per mile, a limit which the agency maintains can be achieved by recirculating some of the exhaust gases through the combustion chamber.

Last week's action was widely expected, and it did not stir up the controversy that Mr Ruckelshaus's earlier decision created, chiefly because few people expect the 0.4 g limit will ever be imposed. The EPA recently released a set of figures to show that the monitoring data which led to the limit being set are incorrect—in 1970, when the Clean Air Act was passed, some 47 urban areas were reckoned to have potentially hazardous levels of nitrogen oxides in their air, but only two cities are now accorded that dubious distinction. Accordingly, the EPA will soon recommend that Congress should amend the act to relax the standard.

One incentive for relaxing the standard is that the motor manufacturers are intending to meet it by installing a second catalyst to car exhausts. The dual catalyst system would carry a large fuel penalty, which is not a good asset with talk of the energy crisis rampant.

It is not yet clear what action Congress will take, but it is likely that a final limit close to the 2 g interim standard set by Mr Fri will emerge. Why is Detroit not happy with it? According to industry spokesmen, one problem they have is that engine conditions desirable for reducing hydrocarbon and

carbon monoxide emissions tend to maximize production of oxides of nitrogen, and so they are not certain that they can meet all the standards at the same time. But it should be remembered that when Mr Ruckelshaus set tough interim standards of hydrocarbons and carbon monoxide, the motor industry chorused that they were impossibly tough, but later decided that they would probably be attainable after all.

ENERGY

Canada's Policy

from a Correspondent

DURING June, the Hon. Donald S. MacDonald, Minister of Energy, Mines and Resources, tabled in the Canadian House of Commons a document entitled *An Energy Policy for Canada*. The government now hopes that the stage is set for a debate on an overall energy policy rather than a consideration of a number of fuel policies as was the case in the past.

Canada is in a unique position among the industrialized nations because its own resources are sufficient to meet current and projected needs. It is thus quite feasible to defer some of the resource development to serve the needs of future generations. There are, however, at least three pressures against this alternative. The first is the desire to increase the cash flow and thus to derive greater economic benefits for the present generation of Canadians. The second is geopolitical. Canada, if it chose this alternative, would be the first non-OPEC country to propose to leave its wealth in the ground. Although Canada now supplies about 22% of USA imports of energy, the rising demands of USA economy would have to be met by oil imports from North Africa and the Middle East, and Canada's relative importance would decrease. Third, there is a real fear that if Canada's oil is not sold now, future energy shortages will force development of alternate supplies (nuclear, solar, geothermal, for example) of energy, and the markets for northern oil will vanish. Irrational as this fear must be, it is based on a still fresh memory of the 1950s when Alberta oil could not be given away which led to the 1961 Energy Policy which prohibited cheap foreign crude oil being imported through eastern ports thus forcing industrialized Ontario to use Alberta oil and gas.

Recognizing the complexity of interlocking constraints, the energy studies document puts a strong emphasis on the requirement for a solid science base. "Priority considerations refer to what is generally defined as research and development. A major priority outside the limits of this specific activity is the im-

provement of our basic knowledge of the extent, accessibility and quality of the resource base. If this basic information is inaccurate or incomplete, all of our planning and policy development is of dubious value."

The document even goes as far as to give a list of priorities for different scientific activities—which is more than a majority of recent science policy studies were prepared to do. The present research effort appears to be lopsided. A preliminary review of federal research and development expenditure on energy in 1971 showed that 71% went into nuclear energy research, while only 11% was spent on oil and gas. As there is no national (state) petroleum company little petroleum research is done in Canada. The report argues at some length that the establishment of a fully integrated National Petroleum Company would automatically improve matters.

In view of the large predicted reserves of oil in Athabasca tar sands (almost twice as much as the total of all other predicted ultimate recoverable reserves of oil and gas in Canada) research on exploitation of this resource is highlighted as the top priority. The extraction at present is only slightly larger than a pilot plant operation and only 0.1% of Federal research and development funds were spent on this in 1971.

The second research priority is to do with techniques and equipment for exploration, production and transportation of hydrocarbons in the Arctic and offshore regions of the continental shelves.

The third priority is the energy resource inventory appraisal. The results of these investigations, however, are critically dependent both on methodology and initial assumptions. Appraisal is further complicated by the fact that inputs from geology, production engineering and market economics must be blended before meaningful estimates can be made.

In the long run the report recognizes the importance of nuclear energy and is reasonably satisfied with the successful design of the CANDU reactor which does not require enriched uranium, but needs heavy water.

The report, however, is conventional and old fashioned in outlook. With its emphasis on growth and continuing expansion of demand, it conveys an impression that the majority of studies were undertaken in the early 1960s, before all the debates generated by Limits to Growth.

At the same time, enough alternative options are raised to give all interested groups an opportunity to voice their opinions and objections. It is to be hoped that it is not just a naive wish that the due regard for the democratic process will give Canadians an energy policy of their choice.

NEWS AND VIEWS

Value of a Hypothesis

PROFESSOR P. A. STURROCK of Stanford University has published a paper on scientific inference in the *Astrophysical Journal* (182, 569; 1973). His object is to evaluate various hypotheses concerning a class of object in the light of several sets of observations upon members of the class, with special reference to applications of astrophysical interest.

Suppose we have a mixed bag of equal large numbers of black and white balls, and suppose two balls are abstracted at random. Consider the hypothesis, H , that these are two black balls. On the background knowledge supplied, the (prior) probability that H is valid is one-quarter. Suppose then that one ball of this pair is looked at and seen to be black; with this additional observational information, the (post) probability that H is valid (the "value" of the hypothesis) is one-half; if one of the pair is looked at and seen to be white, the post probability of H is zero. The theorem giving the general relation between post- and prior-probabilities is Bayes's rule.

In a broad sense, Sturrock produces a generalization of this rule. He illustrates his ideas using the case of pulsars, taking as hypotheses that a pulsar is (H_1) a rotating neutron star, (H_2) a pulsating white dwarf, (H_0) an object of some other sort. What he calls "items" in this example concern pulsed radio emission, range of periods, change of period, association with supernovae, photospheric radiation, power in the case of the Crab pulsar.

The novel feature of Sturrock's treatment is his concept of the "interface" between observation and theory. In any application, the interface consists of several "items". Each item consists of a set of statements about some feature, one and only one of which must be true: in the example the item about change of periods consists of the three statements: all pulsars slow down, all pulsars speed up, neither of these is true. Using the available particular observations, and whatever background information is relevant, the "observer" estimates a probability that each statement is true.

The hypotheses to be evaluated have to form a complete and mutually exclusive set. To them the "theorist" assigns prior probabilities; then he estimates the probability on each hypothesis that each statement in an item is true. The same background information is available to him, but he is supposed to know nothing about the observations special to the problem. Assuming the truth of any statement, and using Bayes's rule, the theorist can then evaluate the (post) probability for each hypothesis. Combining this with the observer's estimate of the probability of each statement, the theorist then obtains the post probability of the hypothesis so far as one entire item is concerned. Sturrock finally gives the (non-trivial) combination of such results for any number of items.

Contact between observation and theory occurs only at the interface of statements. The observer estimates the probabilities that certain fairly general statements are valid in the light of the observational material; as Sturrock says, these estimates must be without theoretical bias. The theorist estimates the probabilities that his hypotheses are valid if one or another of the statements is valid; these estimates must be without observational

bias. Then the two sets of probabilities meet at the interface and produce the evaluations. This formalizes the procedure presumably followed, implicitly and unsystematically, in observational science in general.

In order to follow Sturrock's procedure, the reader needs to study his example on pulsars. He gives it for illustration only, but it is remarkable that some four years ago he got for the post probabilities based on all items: for neutron star 0.995, white dwarf 10^{-8} , some other object 0.005.

Sturrock is disarmingly candid about the several basic difficulties of such work which he explicitly refrains from examining in his paper. In particular, there are two inter-related weaknesses; the need for the "null" or "ignorance" hypothesis (H_0 in the example) and the subjective character of the assigned probabilities.

Significance may be placed upon the probabilities of the other hypotheses relative to the null hypothesis only on the subjective impression that one has been generous to the null hypothesis. As regards the probabilities of the other hypotheses relative to each other, however, under certain circumstances it may be possible to demonstrate that these are insensitive assumptions about the null hypothesis.

In its present formulation, Sturrock's work would apply only to the standard detective-story type of problem where the observer (policeman) and the theorist ("Poirot") have to choose one of several given suspects or the possibility of an "outside job". The detective story-teller's aim is to keep all subjective probabilities about equal until some crucial item comes along. One thing to be learned from Sturrock, however, is that, even if each available item is inconclusive in itself, the resultant effect of several items may give almost completely convincing support for one solution. This would be expected, but it is highly interesting to see Sturrock's quantitative illustration. But again there is still the subjective element, well recognized in detective fiction at the stage when "Poirot" knows the culprit but still has no case for a court of law.

Most astrophysical problems for most of the time are not detective problems of this sort, and the scope for the full application of such work is obviously restricted. Nevertheless it may have value in several ways: first, it may help to classify the activities of various investigators, each of whom may be said to be trying to evaluate any one of Sturrock's probabilities in regard to some current problem, and so to clarify the purposes of the investigations; second, it should show which items of observation might be expected to have most significance for the solution of the problem; third, in a large class of problems, it seems to be the aim ultimately to reach a stage when Sturrock's type of evaluation is needed—implicitly or explicitly—and it may expedite a decision to make it explicit. On the other hand, there are large parts of astrophysics where it would probably be an arid proceeding to try and impose such an analysis. In any event, Sturrock has made a valiant effort to bring order into the haphazard procedures of most astrophysicists.

W. H. McC.

An Alternative to Mantle Plumes

THE bulk of the world's seismic, volcanic, orogenic and large-scale tectonic activity takes place along relatively narrow bands which form a linked pattern around the Earth's surface. The discovery of this pattern played its own important part in the formulation of the new global tectonics; and conversely, it is within the framework of global tectonic theory that the active bands are seen to have physical, rather than simply observational, significance. For the bands define lithospheric plate margins—zones where lithosphere is created, where it finally descends into the mantle, where it collides with other lithosphere, or where lateral motion takes place between plates in what are known as transform faults. In short, whatever else it may do, plate boundary tectonics combines into a single conceptual scheme most of the internal processes changing the face and body of the Earth's lithosphere.

But not all of them. On the ocean floor, volcanism occurs well away from plate margins, very often forming series of mountains and islands, such as the Hawaiian-Emperor chain, which are geographically and temporally linked. Within continental plates are to be found graben, rift valleys and seismic zones. In the United States, for example, a notable series of earthquakes in 1811 and 1812 drew attention to an active seismic zone near New Madrid, Missouri; indeed, the largest known American earthquake outside Alaska took place not in California, Washington or Nevada but as part of the New Madrid series. These and other phenomena may account for a relatively small proportion of the Earth's activity, but they are by no means insignificant and can hardly be dismissed as local.

In view of the influential position now occupied by plate tectonics, it would seem logical to ask how global tectonic concepts may accommodate, or be made to accommodate, important processes taking place away from plate margins. In other words, given the overall framework involving the most significant tectonic phenomena, the obvious next step would be to determine how the less significant (or apparently less significant) processes fit in. Curiously, however, this is not what seems to have happened. For reasons which historians will be left

to discover, many geophysicists have chosen instead to take an inspired, intellectual and, on the face of it, illogical leap to a basically *ad hoc* explanation bearing little obvious relationship to "classical" plate tectonics. And what makes the situation even stranger, in retrospect, is that this new explanation for within-plate processes is now being heralded, in some quarters at least, as the underlying explanation for plate boundary tectonics as well. The child thus becomes father of the man.

The explanation concerned here is, of course, that involving hot spots and mantle plumes. As the debate over the existence of these phenomena is still raging, it is not yet possible to say whether the inspired leap will turn out to have led to a fundamental truth or into a scientific blind alley. In the meantime, however, few would deny that it is highly desirable that alternative mid-plate tectonic theories be put forward for consideration by Earth scientists of all persuasions. Thus whatever one's views on the validity, or otherwise, of mantle plumes, the article by Turcotte and Oxburgh on page 337 of this issue of *Nature* should be welcome.

Specifically, Turcotte and Oxburgh make out a case for explaining mid-plate tectonic phenomena in terms of crustal extension induced by tensional stresses of two types. The important point, however, is that the stresses can be seen as the logical outcome of well-known plate tectonic processes without recourse to any completely new phenomena. If science is to be regarded as a system involving the minimum of unproven assumptions, the new hypothesis put forward by Turcotte and Oxburgh will be regarded as being intrinsically more likely than mantle plumes. Unfortunately, life is not always that simple.

P. J. S.

ORIGIN OF LIFE

Could Life Start Again?

from a Correspondent

THE fascination which the question of the origin of life exerts on scientists as diverse as theoretical physicists, geologists, chemists and biologists of all descriptions, from molecular to ecological, was reflected in the contributions to the Fourth International Conference on the subject held in Barcelona from June 25–

28 under the auspices of the new Autonomous University of Barcelona and the International Society for the Study of the Origin of Life (ISSOL), in order to honour the contributions made to the field by Drs A. I. Oparin (Bach Institute of Biochemistry, Moscow), H. C. Urey and M. Calvin (both of University of California). In the space of 5 days the participants tried to understand what went on from the origin of the Earth (4.6×10^9 years ago) to the end of the Precambrian era (0.7×10^9 years ago) as well as discussing recent and projected interplanetary exploration—a sterile but stimulating exercise.

To set the scene Dr D. Buhl (Radio Astronomy Observatory, W. Virginia) described the recent recognition by radio astronomers of the large clouds of organic molecules in our Galaxy. Many different compounds containing C, H, O and S have been detected but the presence of P is still in doubt. Because of the characteristics of the radio spectrum a particular molecule can be identified with 99% certainty on the basis of a single line. Clouds of organic molecules appear when a star is born, suggesting that the evolution of a molecular cloud is a significant prelude to the origin of life. There seems to be directed chemistry and not chaos in these interstellar clouds. Dr T. Owen (State University of New York) suggested that Titan, the large satellite of Saturn, may provide a model for early organic synthesis and that comets of the outer Solar System with their particular chemical composition may be the link between interstellar space and the formation of the Solar System. The possible formation of organic compounds in interstellar clouds and in meteorites by the Fischer-Tropsch type of synthesis was described by Dr E. Anders (University of Chicago). This type of synthesis can produce most of the simpler and more complex organic carbon compounds from CO, H₂, and NH₃.

The production of diverse organic compounds in abiotic conditions with different types of energy sources, for example electrical discharges, ultraviolet, heat and so on, has been known since the early 1950s and it was their discoverer (Dr S. Miller (University of San Diego, California) who presented a review on the synthesis of amino acids in simulated primitive Earth conditions. Dr J. Oró (Universities of Houston and Barcelona) described prebiotic syntheses of nucleic acid components and oligonucleotides and Dr C. Ponnampertuma (University of Maryland) suggested how small peptides and oligonucleotides may have interacted in the "primordial soup". It was suggested by Dr L. Orgel (Salk Institute, California) that longer oligomers may have been coded for so as to produce alternating hydrophobic and hydrophilic amino acids in a primi-

tive peptide, a process for which Dr M. Paecht-Horowitz (Weizmann Institute, Rehovoth) proposed a catalytic role for specific clay particles.

A highlight of the meeting was the talk by Dr Oparin, who first published his ideas on the origin of life in 1924 and who was undoubtedly a progenitor (along with J. B. S. Haldane, who published later in 1929) of most of the modern ideas in the field. He stressed the multiple character of the origin of life, as opposed to the notion that it was an isolated accident. So called "probiotics" would be formed which undergo a natural selection allowing the survival and development of forms better adapted to the exterior environment. Dr S. Fox (University of Miami) then reviewed the plethora of artificially made proteinoid microspheres which have been considered as models for probiotics. Many simple enzymatic properties have been shown to be inherent in microspheres but now it has been found that proteinoids plus incorporated polynucleotides (protosomes) can condense individual amino acids into peptides. It is proposed that the evolution of information through amino acids and protoprotein to contemporary ribosomal synthesis was continuous. Heterotrophic proteinoid microspheres could have become similar to contemporary types of cells and thus form the link between abiotic organic synthesis and the first type of multifunctional cell. The formation of lipoprotein membranes in the primaevael environment was described by Dr J. F. Danielli (Centre for Theoretical Biology, State University of New York). The primitive membrane could have evolved slowly, utilizing the basic, easily formed lipid bilayer as a model. It is possible that a membrane-associated DNA could have been a primitive genetic system.

Early biochemical evolution was the topic of two colloquia and symposia, which included contributions by Dr M. O. Dayhoff (National Biomedical Research Foundation, Georgetown University, Washington), Dr T. H. Jukes (University of Berkeley, California), Dr D. O. Hall (King's College, London), and Dr H. Baltscheffsky (University of Stockholm) on the evolution of tRNA, 5S ribosomal RNA, cytochrome *c* and ferredoxin based on their known amino acid sequences. So far ferredoxins are the only proteins which have been sequenced and are known to occur in all living organisms investigated. The use of powerful computer techniques is helping the analysis of the sequence data which are now being correlated with biological and physico-chemical information. Dr R. Buvet (University of Paris) showed how compounds which undergo oxidation-reduction reactions, and possibly resembling protein active centres, can be synthesized in condi-

tions simulating the primitive environment. The role of porphyrins, especially chlorophylls, in evolution was stressed by Dr A. A. Krasnovsky (Bach Institute of Biochemistry, Moscow) who has shown the diversity of model and biological electron transfer reactions which can be catalysed by porphyrin-type compounds.

The Precambrian fossil record was well reviewed by Drs J. W. Schopf (University of Los Angeles, California) and M. Muir (Imperial College, London). Discoveries in this field during the past 6-7 years seem to establish that life (bacterial?) probably existed as early as 3.3×10^9 years ago and that by 2.2×10^9 years ago there was oxygen present in the Earth's atmosphere. Some time in this period blue-green algae (prokaryotes) which could evolve oxygen developed, and there are now intimations that multicellular and colonial forms of organisms (possibly algal eukaryotes) may have existed as early as 1.9 to 1.6×10^9 years ago.

Finally, there was a session on planetary exploration and exobiology wherein the 1975 NASA Viking mission to Mars was explained. Biological experiments, calculated to allow for possible misconceptions of the nature of life on Mars, will be conducted to assay *in situ* the possibility of its existence. Dr G. Eglinton (University of Bristol) expressed some optimism for an automatically-returned 1 kg sample from Mars by 1985. In view of all the concern about life in the extreme conditions of space systems and on the primitive Earth, it was salutary to hear Dr W. V. Vishniac (University of Rochester) speak on life in an extreme environment on Earth—to be precise, in a desert called Wright Valley in Antarctica where the average humidity is only 10% and the summer air temperatures are -5 to -15°C . In these severe conditions of lack of water (a few monolayers of water adsorbed on soil particles) some growth and metabolic activity of bacteria and actinomycete-like organisms does occur. Live bacteria were even detected at a depth of 40 cm in the frozen ice. Why go to Mars?

CONTRACTILE SYSTEMS

Primaeval Twitch

from our Molecular Biology Correspondent
APART from relatively minor modifications every few millenia, nature's original model for an engine to turn chemical energy into motion can be adjudged to have survived the caprices of evolutionary fashion. In the most advanced form, the contractile components, actin and myosin, are organized within separate filaments, and are associated also with a system of proteins responsible for modulating activation

by calcium ions injected from the surrounding membrane. In more primitive models there is a less obviously purposeful interaction in an essentially amorphous sludge. The evolutionary pressures have acted almost entirely on the more complex and specialized component of the contractile mechanism, myosin, which differs appreciably from organism to organism, and even from muscle to muscle, whereas the actin is always much the same. As Szent-Györgyi and his colleagues have shown, for example, the myosin in the muscles of the most primitive animals itself embodies the calcium-sensitive control system. The myosin from unicellular creatures, such as the slime mould, is nevertheless remarkably similar in most essential respects to that of mammalian muscles. That of the soil amoeba has now been isolated and characterized, and shows some curious, and so far unparalleled, structural features.

Pollard and Korn (*J. biol. Chem.*, **248**, 4682; 1973) used the potassium-EDTA ATPase, which is unique to myosin, to track down the myosin of *Acanthamoeba*. This activity remained in the supernatant from the homogenates, whereas mitochondrial and membrane magnesium-ATPases were carried down in the pellet. Successive purification steps, involving precipitation and chromatography, led to a substantially pure product, which unlike muscle myosin was soluble at low ionic strength. The ATPase activity was of a similar order to that of muscle myosin, and was inhibited by magnesium but unaffected by calcium. Like muscle myosin the *Acanthamoeba* protein contains heavy chains, and also light chains, two in number and judged by gel electrophoresis in SDS to have molecular weights of 14,000 and 16,000. The evidence concerning the molecular weight of the whole molecule is circumstantial. In SDS electrophoresis the heavy chains come out at 140,000. Pollard and Korn then argue that since the elution volume in a gel filtration experiment on the native protein is no higher than would correspond to a globular protein of about 150,000, rather than to a highly disymmetric species like muscle myosin, such a value must represent an upper limit for the molecular weight. They infer therefore that their protein consists of a single heavy chain of 140,000, with two light chains. It forms no filamentous aggregates, and its amino acid composition has little in common with that of other myosins. The lugubrious response of a protein chemist, confronted with these data, will undoubtedly be to question whether the preparations might represent nothing more than proteolytic fragments of some more respectable myosin-like myosin; he will be bound to concede, however, that Pollard and Korn have made every effort to

eliminate this possibility. They have shown that the properties and the yield of their protein are unaffected by increasing time intervals between homogenization and fractionation, that no ninhydrin-responsive material is generated during the preparation, so that no significant proteolytic activity is in evidence in the homogenate, and that muscle myosin is not broken down on incubation with the same homogenates.

In a companion paper (*ibid.*, 4691) Pollard and Korn go on to characterize the interaction of *Acanthamoeba* myosin with actin. F actin activates the ATPase of the myosin, and in the absence of ATP carries it down when in the ultracentrifuge. In the electron microscope there is little evidence of the formation of the characteristic arrowheads on the actin filaments, perhaps in consequence of the apparently symmetrical shape of the myosin, but the helical repeat of the actin filaments is reinforced and there is increased clustering of the filaments. A unique feature of the interaction is its apparent requirement for a cofactor. This was discovered because at the final stage of purification the actin-dependence of the ATPase activity vanished. The cofactor has been isolated, and is evidently a protein with a molecular weight of some 100,000. Its efficacy is not sensitive to calcium, and there are some unexplained features in the manner in which it operates.

The extensive similarity of the actin of muscle to that of a quite different system, the blood platelet, is demonstrated in a study by Booyse, Hoveke and Rafelson (*ibid.*, 4083). The molecular weight and sedimentation coefficients of the monomeric G actins are the same; both polymerize to F-actin filaments, and these associate in a characteristic manner with muscle heavy meromyosin, both activating its ATPase, the platelet actin albeit to a lesser extent. Structural differences are only apparent in the pattern of peptides generated by chemical cleavage. The presence of F-actin filaments has now also been demonstrated in mammalian (HeLa) cells by Schroeder (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1688; 1973), in the contractile ring which manifests itself in the final stage of cell division. If the cells are extracted at this stage with glycerol, heavy meromyosin can be diffused in, and forms the familiar arrowheads on the filaments, thus identifying them as F actin. If ATP is present this does not occur. Other thin threads interspersed between these filaments are conjectured to contain myosin, so that the contractile ring comprises a genuine muscle-like contractile system.

A further step towards the unification of muscle and other motion-generating systems has been taken by Oplatka and Tirosh (*Biochim. biophys. Acta*, **305**,

684; 1973), who have shown that not only slime mould but also rabbit muscle actomyosin can be made to simulate the cytoplasmic streaming process. When sucked, with a supply of ATP, into capillaries, it gives rise under the microscope to all the disagreeable manifestations — those surging tides of mucus—familiar from films of amoebae on the prowl. The oscillatory changes in the direction of flow can even be observed, if an ATP regenerating system is included to prolong the activity.

HOMOSEXUALITY

Origin of Sexual Drive

from a Correspondent

INTEREST in a disturbance of hormone balance as a cause of homosexuality will be heightened by a report from two workers in California (Margoless and Janiger, *Brit. med. J.*, **3**, 207; 1973). They have shown a significant difference in urinary excretion of male hormones between homo and heterosexual males, but this time using a larger group and aiming at a more accurate psychological assessment than in the earlier work of Margoless published in 1970.

The chief male hormone is testosterone which is largely secreted from the testes, but the adrenal gland also secretes a little testosterone, as well as dehydroepiandrosterone, thought to be a testosterone precursor. Testosterone is metabolized in the liver to androsterone and etiocholanolone. Together with dehydroepiandrosterone these compounds largely make up the complement of 17-ketosteroids excreted in the urine. Taken over 24 hours the average excretion of 17-ketosteroids from a normal male adult is about 14 mg, but in the present study the interest centres on the ratio of androsterone to etiocholanolone excretion over periods of 24 h.

Sixty-three males were studied, the homosexuals coming from various homosexual organizations such as the Gay Liberation Front and the heterosexuals being drawn from college students. Each subject was interviewed by Janiger—a lecturer in psychiatry—and classified using a modified Kinsey sexual inventory into one of six groups from exclusively heterosexual (0–1) through degrees of homosexuality (groups 2, 3 and 4) to predominantly and exclusively homosexual (5 and 6). They were also questioned on their frequency of sexual activity—defined as any activity that resulted in orgasm—and on factors such as parental age and incidence of homosexuality in their families. The subjects underwent a physical examination to detect abnormalities in secondary sexual characteristics and the presence of disease.

After the exclusion of unhealthy

subjects the biochemical studies were done on twenty-three homosexuals, twenty-four heterosexual controls, nine intermediates (Kinsey 2, 3, 4) and one transsexual. There was no difference between homo and heterosexuals in physical factors such as genital size or distribution of pubic hair, or in age of parents at the subjects' birth. Nor was there any difference in frequency of sexual activity between the two main groups. There was, however, a highly significant increase in the incidence of homosexual relatives in the homosexual group.

Biochemically, however, although the total urinary ketosteroids did not differ significantly from normal values in the homosexuals or controls, there was a variation in the ratio of androsterone to etiocholanolone. For twenty-three of the twenty-four controls the ratio was greater than unity, whereas of the fifteen exclusively homosexual subjects of Kinsey scale 6 in only one case was the ratio greater than unity.

As the authors point out, this strengthens support for the hypothesis that the metabolic pathway resulting in high androsterone levels is associated with sexual preference for females by either sex, and that low levels are associated with preference for males by either sex. In other words, the view is that a high androsterone level in males is associated with normal sexual behaviour, whereas in females it is associated with homosexual behaviour. The opposite situation holds for low androsterone levels.

In the past there has been some doubt that the ratios being measured might have been influenced by undetected physical and mental illness in the subjects examined, but the careful physical and psychiatric examination in the present study will have reduced the likelihood of these factors affecting the results.

DIAMOND

High-boron Form

from a Correspondent

THE technological uses of diamond are many and include the cutting of hard materials, rasps, use as a heat-sink, and uses where stability under high temperatures and pressures is required. Theoreticians are also greatly interested in diamond, because it is the simplest analogue of a wide range of technologically-important elements with the same crystalline structure; moreover, the fruits of intensive efforts in theoretical organic chemistry, in which the molecular orbital calculations for carbon-carbon bonds are very advanced, can be passed on for use in the solid-state physics of inorganic materials through this one element.

The physics of diamond has been

arduous and slow to develop because of the difficulty of preparing samples in the laboratory and the high concentrations of impurity found in natural diamond, including nitrogen, aluminium, and boron in the parts per million range. The analysis of impurities is, moreover, confused by the presence in most diamonds (both natural and laboratory-grown) of inclusions which may contribute a large proportion of one impurity. One very interesting form of diamond, known as type IIb, is semiconducting. The forbidden gap of diamond is much wider than for its neighbour in group IV, silicon (about 5.7 as against 1.2 eV respectively) and thus the conduction, which is p-type, must derive from impurities. Activation-energy studies showed that the acceptor level lies at 0.35 eV above the valence band but, because of the abundance of impurities present, the impurity producing this level could not be firmly identified. The hypothesis which was best elaborated was that aluminium is the active acceptor impurity. To fit the data, the picture had to be complicated by interaction with other impurities, namely nitrogen which is well established as a deep-lying donor, and with boron, another acceptor of uncertain energy level, which is also known to be present (Dean, Lightowlers and Wight, *Phys. Rev.*, **140**, A352; 1965).

This hypothesis, although fairly widely accepted for a time, was not universally accepted (Wedepohl, *J. Phys. C*, **1**, 1773; 1968) and finally Collins and Williams (*J. Phys. C*, **4**, 1789; 1971) presented the idea, based on more accurate analyses of aluminium in diamond, that boron is a more likely candidate for the dominant impurity. This new proposal was generally accepted by workers in the field, especially because it had the support of some unpublished data of Chrenko, of General Electric Research Laboratories. Chrenko has now released what might be regarded as the largest mass of authoritative data yet available on the subject. He concludes that boron must indeed be the dominant acceptor. General Electric Corporate laboratories are the leaders in the synthesis of diamonds large enough for this kind of research and are one of the few centres in the world where such diamonds with well-controlled amounts of impurity can be synthesized. Writing in *Physical Review* (**B7**, 4560; 1973), Chrenko concludes that there is a good correlation between boron content and acceptor concentration, but aluminium content, intentionally kept low, does not correlate well. What is more, the acceptor concentration is considerably higher than the total aluminium content as determined by neutron activation analysis, thus rather firmly excluding aluminium from con-

tention as the dominant acceptor, although other potential acceptors, for example beryllium, are not as rigorously dealt with.

Considering the previous difficulty in determining boron content (in natural diamonds of the usual experimental size, the total mass present is so low as to be near the limit of detection by any method), the data presented are unique and represent an important new acquisition of knowledge. Chrenko also improves on the moment by reviewing carefully the previous principal hypotheses and thus sets the new data firmly in the framework of the old.

There are, of course, reservations which should be made: the acceptor concentration was estimated from optical absorption rather than by electrical means and the author also admits that the distribution of impurity was not uniform, but showed a reticular pattern. The chief uncertainty in this work is always the unfortunate presence of metal-rich inclusions in many samples, which throws off the calculation of bulk impurity concentrations from bulk analytical data, and caused the original false impression concerning abundance of aluminium. Finally, aluminium has only been demoted, and not eliminated completely as an active acceptor impurity. It is probably true to say, however, that this first report on new, large, high-boron, low-aluminium diamonds gives only a foretaste of the good basic work that can be done when they are more thoroughly studied by a wider range of workers. While it is unlikely that diamonds will be used widely as semiconductor devices, this form of diamond represents a very interesting tool in the study of extrinsic conduction in solids.

RECLAMATION

Changing the Spoil Heap

from our Plant Ecology Correspondent
NATURE is, on the whole, well equipped to cope with calamities, even when they involve the complete destruction of life in an area, for example, following glaciation or volcanic eruption. As soon as the interfering factor is removed, the process of biological succession is initiated in which progressively more complex communities replace one another until eventually a stable climax state is attained. Even most man-made catastrophes are followed by these natural repair processes, but there are certain exceptions which present pressing problems to ecologists concerned with the restoration of over-exploited environments.

One example of a situation in which natural successional processes are inhibited is provided by spoil heaps in which high concentrations of toxic residues

remain. There are several reasons why efforts should be made to hasten the process of succession in such situations. In the first place there is the amenity consideration which, although important, is difficult to evaluate objectively. Second, there is the problem of erosion of toxic materials and their redistribution; and, third, there is the possibility of soluble toxins reaching rivers and aquifers from which they might find their way into human tissues. These problems can all be alleviated by encouraging the growth of vegetation on spoil heaps because this will stabilize the material and also reduce run-off water by increasing evapotranspiration.

Breeze now describes (*J. appl. Ecol.*, **10**, 513; 1973) some studies he has made on spoil heaps resulting from chromium extraction processes in the Croal Valley, Lancashire. The reclamation of these waste heaps was proposed by the Lancashire County Council both on the grounds of amenity and in the hope of improving the river Croal. Breeze found that the diversity of algal and macrophyte vegetation of this river is considerably reduced in the region below the spoil heaps.

The waste is the product of various processes, the chief of which was the extraction of chromate salts from chrome iron ore, $\text{FeO} \cdot \text{Cr}_2\text{O}_3$. The ore was roasted with sodium carbonate and lime and the resultant sodium chromate extracted. About 5% of the ore was converted to calcium chromate, however, and as much as 10% of the original ore remained unaltered. Additional waste products included calcium aluminate, calcium hydroxide, magnesium hydroxide and ferric oxide, producing a residue with a pH of 12.5. This material was accumulated during the period 1880 to 1968 as spoil heaps up to 15 m high.

The spoil in its present form is eminently unsuitable for plant growth. A reduction in pH, however, would increase the solubility and hence the availability of chromium, aluminium and magnesium, all of which are present at toxic concentrations. If the pH were reduced to 7, the amount of chromium entering solution would be increased five times.

Breeze has conducted experiments on the toxicity of chromium in its anionic and cationic forms and has found that the chromate anion is toxic at far lower concentrations than the chromic cation. Potassium dichromate is toxic to *Lolium perenne* at 500 mg Cr kg^{-1} soil, whereas chromic sulphate had to be supplied at a level of 5,000 mg Cr kg^{-1} soil to produce equivalent toxicity. Chemical reduction of chromate to chromic ions might therefore alleviate toxicity and permit invasion of the heaps by vegetation.

An alternative technique is to cover

the spoil with a layer of soil. This should be of neutral or alkaline reaction, otherwise its leachate would dissolve more chromium from the underlying spoil. Upward diffusion of chromium would remain a problem; this has been shown to occur through 15 cm layers of soil. An impermeable layer between the spoil and the soil would lead to erosion of the upper soil. It seems, therefore, that chemical reduction of chromate is the best hope for a long-term solution to the problem of reclamation of chromium waste heaps.

EARTHQUAKES

Predicting Locations

from our Geomagnetism Correspondent

EARTHQUAKE prediction must obviously involve both the times and locations of impending events. But having acknowledged that, it is possible to visualize two rather different approaches to the prediction problem with rather different emphases on time and place. It is probably true to say that prediction has most often been perceived as the problem of determining in advance the times of events likely to occur in regions known to be seismic, and in such cases the seismic regions may be regarded as essentially local even though some may have similar characteristics (similar fault types or similar earthquake depth ranges, for example). Thus attempts to predict earthquakes by seeking, for example, premonitory geodetic, geomagnetic or seismic velocity changes have been carried out in specific areas, certainly in the hope that any conclusions emerging may be applicable to other seismic zones, but more realistically in the expectation that the results may be applied at least within the particular region concerned.

Clearly this approach is possible even in the absence of any general framework linking all, or most, seismic zones. Japan, for instance, has long been known to be seismically active, and attempts have been made to predict the times of occurrence of earthquakes since the last century. But the advent of the new global tectonics has opened up a new approach which may be expressed as attempting to deduce, not so much the times of impending events in a particular area, but rather the likely locations of events within a particular time interval. This possibility first became apparent in a general way with the discovery that seismic zones are not randomly scattered about the world but form a series of linked belts now known to correspond to plate boundaries (although some earthquakes also occur outside these belts). At this point it became possible to make a statistical statement to the effect that future events are much more likely to take place

along the defined belts than in the interiors of the plates. Moreover, more detailed work on the nature of plate boundaries led to more detailed predictions about where particular types of earthquake would occur—for example, that very large earthquakes are much more likely to occur along subduction zones and along transform faults than along oceanic ridges.

But can this approach be refined? In other words, given that future earthquakes are more likely to occur along the defined belts, can the belts themselves now be subdivided into segments in which the chances of an earthquake occurring during a specified period differ? For although this may not lead to precise prediction in the sense of being able to forecast the exact time and place of a future event, it would nevertheless be of some social importance to be able to predict whether, say, the San Francisco area is very likely or highly unlikely to experience a significant seismic event during the next few decades.

In a long and detailed analysis of the Pacific plate margins, Kelleher *et al.* (*J. Geophys. Res.*, **78**, 2547; 1973) have now shown that it is indeed possible to subdivide the circum-Pacific seismic belt in this way, although they admit that this first systematic attempt is "crude" and that "profound social changes" are therefore not warranted. Their chief aim was to predict the locations of large (magnitude ≥ 7) shallow earthquakes due to take place over the next few decades around the Pacific; and they have done this by successively applying two sets of criteria to give a three-fold classification of marginal segments.

The initial set of criteria was designed to exclude all segments which are not part of a major shallow seismic belt characterized predominantly by thrust or strike slip faulting, and all segments which have ruptured during the past 30 years as a result of large earthquakes. The object of the first of these criteria was largely to remove from consideration those regions not relevant to the principal aim and thus to exclude intermediate and deep earthquakes, large shallow earthquakes within plates and earthquakes associated with normal faulting. Spreading ridges were also eliminated because large shocks rarely occur along them, and a few other areas were removed because of tectonic complexity. The point of the second criterion is that there is now considerable theoretical and observational evidence to suggest that large earthquakes are least likely to occur in the immediate future in zones which have experienced such events in the recent past. In short, there is a tendency for large earthquakes to fill in the "seismic gaps" which have not been subject to

rupture for some considerable time. This was first appreciated by Fedotov (*Tr. Inst. Fiz. Zemli Akad. Nauk SSSR*, No. 36, 66; 1965) and was used by him and others to predict earthquake locations in limited areas. Thus Kelleher *et al.* do not claim that their initial criteria are new, although this is the first time they have been systematically applied over such an extended region.

To the areas of "special seismic potential" defined by the initial criteria, Kelleher and his colleagues then applied a supplementary set of three criteria in an attempt to pick out the zones most at risk to large earthquakes during the next few decades. According to the first supplementary criterion, a historical record of one or more large events along a segment is regarded as evidence that similar events can occur there again. If no such record exists, it may be that the strain is being released by aseismic creep or small to moderate earthquakes, and the risk of a large earthquake will be low. On the other hand, it could also be either that the historical record is incomplete or that the recurrence time for large earthquakes is longer than the historical record. This criterion is therefore not foolproof.

The point about the recurrence interval is taken up again in the second criterion, for an area is considered to be particularly at risk in the near future if historical data and relative plate motions suggest that the recurrence time is comparable to the time since the last large earthquake occurred. But although this is straightforward enough in principle, calculated recurrence times are particularly vulnerable to defects in the past record of events and variations in relative plate motions and will thus be subject to large uncertainties. Finally, an area is designated as one of high risk if it appears to be the site of the next event in a series of earthquakes progressing regularly in time and space. There are now several recorded examples of regular progressions of events along extensive sections of plate boundaries, suggesting that many earthquakes are causally related.

By systematically applying the various criteria in detail to each segment of the Pacific belt, Kelleher and his colleagues end up with a map defining three degrees of risk with respect to large shallow earthquakes. The low risk areas are those excluded by the initial set of criteria. The risk in those areas not so excluded is higher, and is higher still in those regions selected by one or more of the supplementary criteria. But as the authors point out, the uncertainties are large. They thus regard their results not as the last word but rather as "working hypotheses to be tested, modified, and discarded if necessary".

LIQUIDS

Water Comes into its Own

from our Molecular Physics Correspondent

THE structural thermodynamics of water, which in the forty years since the classic paper of Bernal and Fowler (*J. chem. Phys.*, **1**, 515; 1933) has so often been a matter of inspired geometrical guessing, shows recent signs of an important fruition which may bring it, if not to the exalted status of the theory of simple liquids, at least to a state in keeping with its general language and methodology. Two very substantial articles (Weres and Rice, *J. Am. chem. Soc.*, **94**, 8983; 1972; Bell, *J. Phys. C*, **5**, 889; 1972) mark this trend, to which a contributory factor has undoubtedly been the appearance of the first convincing computer simulation of a real water model (Rahman and Stillinger, *J. chem. Phys.*, **55**, 3336; 1971; see also *Nature*, **244**, 256; 1973).

As has been stressed on previous occasions (see *Nature phys. Sci.*, **230**, 74; 1971) the available evidence on the structure and dynamics of water from both "ordinary" measurements (density, viscosity, calorimetry) and more sophisticated probes (infrared spectra, neutron and X-ray scattering, nuclear magnetic resonance, dielectric relaxation) seems at times as confusing in its variety as equivocal in its implications. Moreover, the central feature of any theory of water—the hydrogen-bond—remains lamentably ill-defined and can be expected to be the centre of quantum-chemical controversies for some time to come.

Several particular ingredients have combined to make the new models of Weres and Rice and of Bell particularly effective in the present state of knowledge. One is the recently improved potential of Ben-Naim and Stillinger (*J. chem. Phys.*, **52**, 5531; 1970) which, following an earlier proposal by Bjerrum, treats the H_2O molecule as a tetrahedron of positive and negative charges supported on a repulsive core. Another is the recognition that the lattice-gas model, though somewhat discredited in the theory of simple fluids, comes into its own in the case of water, whose complexity still places it somewhat beyond the reach of the more popular integral-equation methods, such as the Percus-Yevick approach.

Given the original Bernal-Fowler picture of an essentially tetrahedral pattern of H-bond linkages, the appropriate lattice cell for the modelling of short-range order is almost unquestionably the partially-occupied body-centred cubic arrangement characteristic of ice-I, within which the H-bonds can "flip" between a well-defined and limited number of configurations, consistent with the so-called "Pauling rules".

Although other authors have attempted to explain the temperature-dependence of density in terms of competition with the denser ice-II structure, the computer experiments, at least, seem to rule out this possibility for normal temperatures and pressures.

Although both Weres and Rice and Bell share the ice-I lattice as starting point and perform similar analyses of the combinatorial statistics of the water molecules in their lattice positions, their approaches, in fact, diverge considerably. Bell, perhaps closer to the spirit of lattice theories, develops his model in terms of two basic parameters, a "bonding-energy" and a "repulsive energy", while Weres and Rice follow through the implications of the BNS potential in great detail, grafting onto their basic lattice model ever more ingeniously-justified elaborations—a quantum harmonic approximation for librational motions, a dielectric continuum to account for inter-cell perturbations, an empirical expansion coefficient to fix the cell dimension, communal entropy taken as proportional to the fraction of intact H-bonds, and so forth, to mention only a few. Although at times they come dangerously close to obscuring the wood by the trees, the effect, after some twenty pages, remains a fine balance of cunning and insight, and the results, a handful of figures giving the various contributions to enthalpy and entropy at 0° and 100° C, are no anti-climax. Though the Weres-Rice results for H and S are not spectacularly close to experiment, they are, by their own high standards, credible, and, moreover, contain useful subsidiary information, for example as to the importance of quantum contributions to the librational motion. Bell, whose approach is overall somewhat less ambitious, rests content that he is able to predict successfully the density maximum, its disappearance at a supercritical pressure, and the observed minimum in isothermal compressibility.

Taken in isolation, both these attempts to reach quantitative thermodynamic conclusions might seem open to the charge of squeezing too much out of an unpromising and grossly underdetermined physical situation. Both undoubtedly contain an unsatisfactory element of seeming to be "designed" to give the type of results by which the authors set particular store—P-V-T data for Bell, S-H-T data for Weres and Rice; neither seems to offer even the remotest chance of predicting a phase transition without very considerable refinement. Nevertheless, an altogether more promising complexion is given to the problem by the Rahman-Stillinger computer simulations which Rice and Weres have

gone to some lengths to consider.

The "molecular dynamics" approach, though still far short of computing the all-important entropy of the system, nevertheless provides (quite literally) an accurate picture of the short-range order and dynamical correlations which follow from the adoption of a particular potential model. Even with the rather restricted samples so far generated (~400 molecules in the most recent work), it now seems clear that several of the wilder guesses as to the nature of the short-range order can be ruled out. Thus, although distorted tetrahedral networks of strained H-bonds are a dominant motif, there seem to be no recognizable "clusters" or regions of anomalous density beyond ordinary thermal fluctuations. Furthermore, although the bonding system seems reasonably well-defined and "dangling" H-bonds can be seen, there seems to be no separation into "network" and "interstitial" molecules and no evidence of interpenetrating networks of the ice-VII and ice-VIII such as required by some alternative lattice-type theories (see, for example, Perram, *Mol. Phys.*, **21**, 1077; 1971). Finally, so far as the dynamics can be interpreted, diffusion seems to occur by strongly coupled motions with no evidence of "jumps" from one relatively long-lived configuration to another. All this undoubtedly adds up to a picture which, at the short-range level at least, is not too far removed from the severely idealized models which Bell and Weres and Rice adopt.

The chief casualty in these findings is, of course, the class of theories whose authors put their faith in the existence of elusive "cooperative effects" in the formation of neighbouring H-bonds, the most picturesque and persuasive of these being the "flickering cluster" model of Frank and Wen (*Discuss. Faraday Soc.*, **24**, 133; 1957). This and other theories involving substantial concentrations on "non-H-bonded" water have already been under fire from infrared spectroscopists (Stephenson, *J. chem. Phys.*, **69**, 2145; 1965), and their passing will surely cause a sigh of relief from theoreticians who, so far from welcoming the simplicity of the appeal to "cooperative effects", see in their radically N-body character something more like a counsel of despair.

Perhaps a large part of the fascination of the water problem lies not so much in its biological importance, or in the judicious rendering of uncertain structural tendencies into soluble mathematics, or in the actual mechanics of what are undeniably tedious calculations—but rather in the exasperating nuances of significance in the process of arriving at what might be called a "best fit" to experimental results drawn from almost every type of physical measurement possible on condensed matter.

Embryo Transfer and Sensitivity to Teratogenesis

C. R. AUSTIN

Physiological Laboratory, Cambridge

This article was submitted at a working party jointly set up by the British Association for the Advancement of Science and *Nature* to study the scientific, legal, political and religious implications of recent advances in biology that increase the possibilities for the control of human development and heredity.

MORE than 80 years ago, Heape¹ recovered two four-cell embryos from one rabbit and placed them in the oviduct of another which had been mated three hours previously. In due course the recipient rabbit (Heape used the term "uterine fostermother") gave birth to six young, four resembling herself and her mate while two had the fur characteristics of the donor rabbit.

Since that time many articles have appeared on the successful outcome of transfer experiments, involving ovarian or freshly ovulated eggs (for fertilization in the recipient or *in vitro*), eggs undergoing fertilization, or cleavage embryos up to the blastocyst stage. In 1971, Adams and Abbott² published a bibliography listing 451 original articles on egg and embryo transfer; in addition there were sixteen theses and forty-one books and reviews in which the subject was dealt with. The experimental work involved fourteen different species distributed over six Orders of mammals (rabbit, rat, mouse, hamster, guinea-pig, ferret, mink, sheep, goat, cow, pig, deer, monkey and wallaby). Observations included rates of implantation, birth and foetal mortality, and foetal and neonatal anomaly.

This material represents a considerable body of observation and experience from which two points emerge very clearly. The first is the high frequency of success that can be obtained with egg or embryo transfer in the various animals used. Now that specific requirements are appreciated—in particular that the endocrine status of the recipient be appropriate for the stage of embryo transferred—the establishment of pregnancy and in due course birth of young generally follow. The second point is the rarity of birth defects. Indeed, there does not seem to be a single report in the literature in which any of a great variety of procedures could significantly or even suggestively be linked with increase in incidence of birth defects above the normal level. Prenatal losses certainly occurred, often more frequently than in natural conditions; sometimes they involved late stages of development but they chiefly happened before implantation. Treatments have included: (a) fertilization *in vitro* under many different conditions; (b) exposure to culture media of widely varying composition, pH and osmolarity, and to environmental temperatures between 0°–40° C for different times, (c) storage of two-cell and eight-cell embryos for many weeks at –269° C; (d) separation requiring development from single cells from two-cell, four-cell and eight-cell embryos; (e) transfer of sheep or cow embryos to rabbit recipients for several days before insertion in a sheep or cow recipient; (f) biopsy of part of the tropho-

blast in blastocysts; (g) fusion of morulae in pairs or larger aggregates; (h) dissection at the blastocyst stage—bisection to form two blastocysts, exchange of inner cell mass between blastocysts or injection of additional cells from other blastocysts.

The implications of these facts can be brought out more forcibly by looking at some of the research on causes and mechanisms of production of congenital abnormalities. In the course of many investigations attempts have been made to produce birth defects by treating the embryo or the mother during the preimplantation phase of pregnancy. In a few instances positive results have been claimed, but generally the reports are clearly open to other explanations than that involving a direct action on the embryo. For example, Werthermann and Reiniger³ noted ocular defects in a few rats (about 12%) whose mothers had been subjected to hypoxia in the first week of pregnancy—they could have been due to a detrimental effect on the maternal system. Smith⁴ froze female hamsters for 30 to 45 min on different days of pregnancy from 0.5 d to 12.5 d, and killed them just before term. In the litter from an animal treated for 45 min at 2.5 d of gestation all foetuses were "grossly malformed"—none of the other eleven animals frozen in the preimplantation period gave such a result, which could well have been genetically determined. Rugh and Grupp⁵ described an increase in exencephaly in the young born to mice irradiated between 0.5 d and 1.5 d after ovulation, but their "control" animals were not examined at the same time, nor sham operated, nor were they known to be comparable genetically, in age or parity; others⁶ could not confirm these results with similar treatment. Morris⁷ injected pregnant rats with large doses of vitamin A and examined the litters after birth. In eight young rats, among 144 developing in mothers that had been injected just before the time of implantation (3.5 to 4.5 d after mating), there were malformations of the central nervous system—again probably due to changes in the maternal organism. Lin and Monie⁸ reported finding haematomas in three out of thirty-five mouse foetuses developing from blastocysts that had been cultured in 0.5% trypan blue solution or inoculated with a 0.1% solution of this chemical agent, before transfer to recipients. Trypan blue is known to enter blastocysts readily and to persist for many days. Other cases are discussed by Kalter⁹.

The remarkable resistance to teratogenesis shown by preimplantation embryos stands in striking contrast to the response that can be elicited from embryos shortly after implantation, during the phase of organogenesis. An impressive range of agents—irradiation, chemical mutagens (including trypan blue), penicillin, aspirin, alcohol, thalidomide, hormones, vitamins A and D, rubella, dietary deficiencies, hypoxia and so on—can produce birth defects, often at a high incidence and even reaching 100%, when applied at this time. The explanation of the difference seems to be essentially simple. The cells of the early cleavage embryo possess in large measure the capacity to form any part or even the whole of the future individual¹⁰, as they have not undergone differentiation and so losses of cells can to some extent be compensated for. The principal effects of teratogenic agents seem to be to kill cells or prevent their multiplication. The effect on the cleavage embryo depends on the number of cells killed or inhibited:

above a certain proportion, the embryo dies; below that figure, the remaining cells multiply to replace those lost and subsequent development is essentially normal. Even with the formation of the blastocyst following its differentiation into two types of cells, some such adjustment is possible because the precise fate of inner-cell-mass cells (from which the new individual develops) is still undecided¹¹. Shortly after implantation, however, differentiation proceeds apace and cells are set aside for the formation of the various tissues and organs. Cell death on any significant scale during organogenesis cannot be made good, and the results are permanent structural and functional defects.

A brief word should be said about chromosomal anomalies. Inappropriate culture media and the ageing of eggs before fertilization can interfere with egg maturation, the response of the egg to sperm penetration and the cleavage of the fertilized egg, and thus increase the normal incidence of triploidy and tetraploidy in embryos. These states are highly lethal, nearly always resulting in early embryonic death; they characterize a large proportion of spontaneous human abortions. It is possible for triploid fetuses to survive to birth, and for shortly thereafter, but only as extremely rare events^{12,13}. In

most of these instances survival seemed to have depended on the fact that some tissues comprised diploid cells; the mode of origin of these fetuses is obscure.

- ¹ Heape, W., *Proc. R. Soc., B*, **48**, 457 (1891).
- ² Adams, C. E., and Abbott, M., *Bibliography No. 45* (Reproduction Research Information Service Ltd, Cambridge, 1971).
- ³ Werthermann, A., and Reiniger, M., *Acta Anat.*, **11**, 329 (1950).
- ⁴ Smith, A. U., *J. Embryol. exp. Morph.*, **5**, 311 (1957).
- ⁵ Rugh, R., and Grupp, E., *J. exp. Zool.*, **141**, 571 (1959).
- ⁶ Russell, L. B., in *Preimplantation Stages of Pregnancy* (edit. by Wolstenholme, G. E. W., and O'Connor, M.) (Churchill, London, 1965); Brent, R. L., and Bolden, B. T., *Radiat. Res.*, **36**, 563 (1968).
- ⁷ Morriss, G. M., thesis, Univ. Cambridge (1971) (*J. Embryol. exp. Morph.*, in the press).
- ⁸ Lin, T. P., and Monie, I. W., *J. Reprod. Fert.*, **32**, 149 (1973).
- ⁹ Kalter, H., *Teratology of the Central Nervous System* (University of Chicago Press, Chicago and London, 1968).
- ¹⁰ Moore, N. W., Adams, C. E., and Rowson, L. E. A., *J. Reprod. Fert.*, **17**, 527 (1968).
- ¹¹ Gardner, R. L., *Adv. Biosci.*, **6**, 279 (1970).
- ¹² Böök, J. A., Masterson, J. G., and Santesson, B., *Acta genet. statist. med.*, **12**, 193 (1962).
- ¹³ Schindler, Anne-Marie, and Mikamo, K., *Cytogenetics*, **9**, 116 (1970).

Early and Late Methylations in HeLa Cell Ribosome Maturation

M. SALIM & B. E. H. MADEN

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ

Early and late methylations of HeLa cell rRNA occurring during maturation of the rRNA have been characterized.

KNOWLEDGE¹⁻⁵ of the methylated sequences within a mammalian rRNA and its nucleolar precursors is of structural interest, and may help to clarify the role of RNA methylation in mammalian ribosome maturation. We present here an analysis for HeLa cell rRNA.

It was thought that all methylation of mammalian rRNA occurs rapidly in the nucleolus on 45S RNA¹⁻³, but more recent results using HeLa cells⁶⁻⁸ indicate that at least a few 18S methylations occur later during maturation. It is therefore important to distinguish clearly between many early methylations and a few late ones. This was achieved in the experiment shown in Fig. 1 which demonstrates the late methylation of three 18S products, 30, 34 and 49, absent in T₁ fingerprints of nucleolar RNA⁷. One faint, hitherto unnoticed, 28S product (2a) is also labelled but no other products are labelled. On the basis of this experiment, and from further comparison of ribosomal with nucleolar methyl fingerprints, we conclude that all other methylations occur early during ribosome formation. This early phase of methylation probably commences on nascent 45S RNA¹.

Methylated Oligonucleotides

To characterize the methylated products which arise in T₁ digests of rRNA, we used standard T₁ fingerprints⁷ (Figs 1 and 2a), and "T₁ plus phosphatase"⁹ fingerprints with long separations in both dimensions for resolving uridylylate rich products (Fig. 2b, c and d).

Sequence analyses were carried out by a combination of procedures using both ¹⁴C-methyl-labelled and ³²PO₄-labelled rRNA, after first locating methylated oligonucleotides in ³²P fingerprints by a double isotope method. The complete sequences of many products were obtained. For other products, mainly large ones or those which could not be obtained pure from complex regions of ³²P fingerprints, only characterization of methylated components within the oligonucleotides was carried out. Confirmatory identifications were carried out on many products from 32S and 45S RNA. Quantification of all products was performed on at least two, and usually three, ¹⁴C-methyl fingerprints, made by both T₁, and T₁ plus phosphatase, methods, on each of the four main classes of RNA (18S, 28S, 32S and 45S). The main findings, to be reported in full elsewhere, were as follows (Table 1).

28S RNA contains some seventy-one methyl groups distributed between fifty-four chemically distinct T₁ products. Most products are singly methylated and were recovered in molar yields of close to unity ($\pm 15\%$). A few products are multiply methylated, two of the smallest products occur several times

per mol, and a very few products occur in clearly fractional molar yields. Of the methyl groups, all except five, which seem to be base methylations, were characterized as 2'-O-ribose substituents. 18S RNA contains some forty-five methyl groups. Most 18S methylated products are different from the 28S products, but a few small products are common to both RNA species⁷. As with 28S RNA, most 18S products were recovered in molar yields close to unity, though there were a few exceptions. Three 18S products contain more than one methyl group (Table 1). One of these is the strong late spot 30 which possesses the sequence, $m_2^6Am_2^6ACUG$, and is very similar to both a late methylated sequence in yeast 17S RNA¹⁰ and to one near the 3' end of *Escherichia coli* 16S RNA¹¹⁻¹³. The two other late 18S methylations are also base methylations but all the early 18S methylations were characterized as ribose methylations, with one possible exception (spot 69) which contains a still unidentified component.

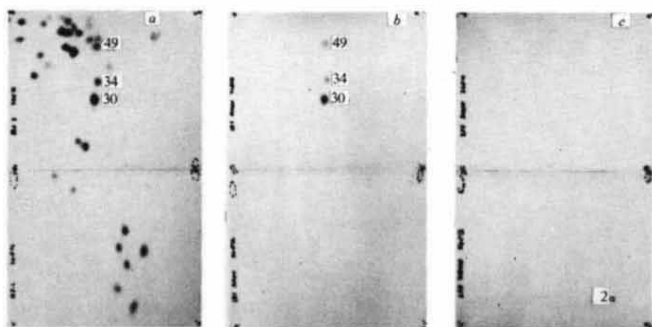


Fig. 1 Late methylations on ribosomal RNA. Two rapidly growing monolayer cultures of HeLa cells, each with 10^8 cells, were incubated with $50 \mu Ci$ ^{14}C -CH₃-methionine. For controls, Eagle's medium was replaced by 50 ml methionine free medium with 5% dialysed serum, 2×10^{-5} M adenosine and guanosine and 10^{-2} M Na formate⁷. $50 \mu Ci$ (1 μmol) of ^{14}C -CH₃-methionine was immediately added and the cells were incubated for 90 min. For the other experiments actinomycin D (4 U ml⁻¹) was added immediately after changing the medium, and ^{14}C -CH₃-methionine 5 min later. Cells were collected, cytoplasmic rRNA prepared, and fingerprinted after T₁ digestion as described⁷ with one modification—because of the large quantity of RNA required to yield adequate radioactivity after the short labelling period (about 300 μg of 18S RNA and 800 μg of 28S RNA), 'CelloGel' (Reeve Angel) was used instead of cellulose acetate for fractionation in the first dimension of the fingerprints. a, 18S control; b, 18S with actinomycin; c, 28S with actinomycin.

The numbers of methyl groups calculated from this analysis (Table 1) agree well with the numbers recently estimated by a different method, based on the determination of the absolute molar yields of several methylated products in combined T₁ plus pancreatic RNase digests of ³²P-labelled RNA⁸. The combined results of these analyses are not consistent with the possibility of extensive duplication of the methylated sequences in HeLa rRNA, as was previously reported for *E. coli* 23S RNA¹⁴. Nevertheless, the numbers of methyl groups reported here are considerably higher⁸ than those estimated previously for HeLa rRNA³.

A careful comparison was made between rRNA and the precursors, by both T₁, and T₁ plus phosphatase, fingerprints, to test the supposition^{3,4} that methylation is confined to the ribosomal sequences of the precursor molecules. There was almost complete correspondence between both the 28S and 32S RNA and the 28+18S and 45S RNA methylation patterns, apart from the absence of known late methylated spots in nucleolar fingerprints. There were a few very minor discrepancies between 45S and rRNA but these seemed to be largely if not entirely attributable to variable incomplete methylation of a few products within 45S RNA. Otherwise the data provide the most direct evidence yet obtained that

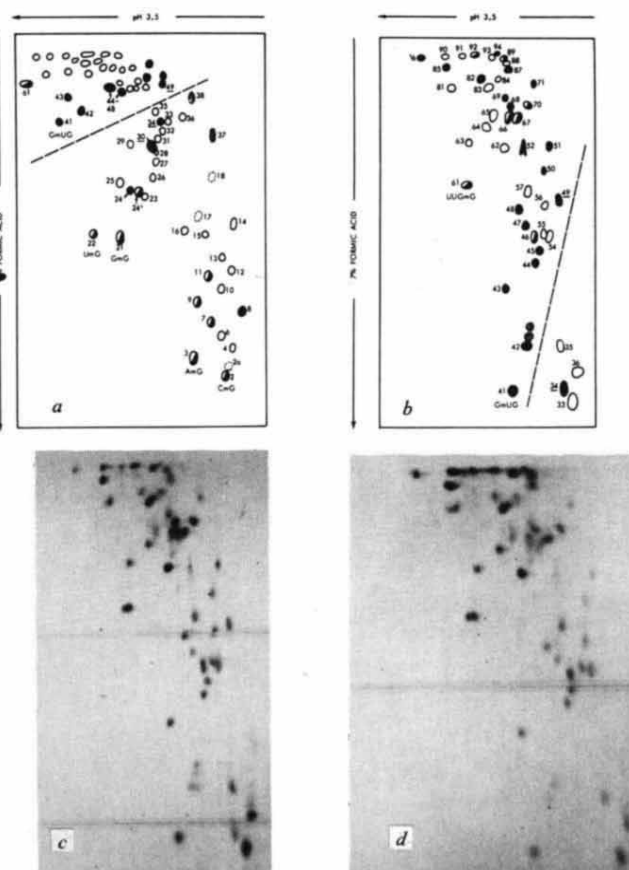


Fig. 2 a, Standard T₁ and, b, T₁ plus phosphatase keys to CH₃ of HeLa cell rRNA⁷, and T₁ plus phosphatase fingerprints, of, c, 28+18S and, d, 45S RNA. T₁ plus phosphatase fingerprints⁹ were made using the following separation conditions. First dimension, cellulose acetate, pH 3.5, 4.5 kV for 5–6 h; second dimension, DEAE paper, 7% formic acid, 1.3 kV for 36 h. ○, 28S unique spots; ●, 18S unique spots; ◐, spots common to 28S and 18S RNA. Faint spots are indicated by dotted circles (28S, open; 18S, shaded). Products below the interrupted line were generally fractionated by T₁ fingerprints, those above the line by T₁ plus phosphatase fingerprints. Overlapping 28S and 18S spots do not necessarily signify sequence identity. Several of the smallest products are common to both rRNA species, but several other overlapping 28S and 18S spots yield different products on further degradation. Note the absence of spots 34 and 49 in the 45S fingerprint (spot 30 (2c) has run off). The RNA used in these fingerprints was prepared as described previously⁷.

methylation of 45S RNA is essentially confined to its ribosomal sequences.

Early and Late Methylations

45S RNA thus undergoes rapid methylation in the nucleolus at some 110 points within its ribosomal sequences. Most methylations are on different primary sequences, and all except five or six are ribose methylations.

Later, during maturation, six base methylations occur on 18S RNA, and one very minor one occurs on 28S RNA. Four of the late 18S methylations give rise to the product, $m_2^6Am_2^6A$.

The large number of different primary sequences which undergo ribose methylation suggests that the specificity for this type of modification resides in secondary structure rather than purely in primary structure within 45S RNA^{15,16}. If so, the secondary structures of ribosomal and non-ribosomal sequences must differ substantially from each other, as the latter are not methylated.

That the great majority of methylated products were recovered in molar yields of close to unity (Table 1) suggests

Table 1 Methylated Products in HeLa Cell rRNA

28S	Spot No.	Methylated component (or sequence)	CH ₃ groups mol ⁻¹ RNA Observed	Suggested
Singly ribose methylated products	All except those listed below:		48-49	
Multiply methylated and/or base methylated products	2a *	mCG	Faint	0.25 ?
	14	mA; ... AmC or CmA ...	1.60	1.5-2
	28	U(A,mA)CG	0.97	1
	33	mC; C,AAAmU,G	2.22	2
	36	AC ₃₋₅ GmAAmAG	1.76	2
	62	CmAGmUUG	2.21	2
	63	mUUψAG	1.18	1
	67.28	mC, ... 2AmU ...	2.51	~3
	83	... UmGmU ...	1.93	2
	90	... GmU,UmC,GmAAC ...	2.53	~3
	92a	... UmGmw ...	1.83	2
	93	... (A ₂ X)U ...	1.63	2-3 ?
Sum of 28S CH ₃ groups				~71(±1)
Previous estimate ^a				~68
18S				
Singly ribose methylated products	All except those listed below:		35	
Multiply methylated and/or base methylated products	30 *	m ₂ ⁶ Am ₂ ⁶ ACUG	3.67	4
	34 *	... m6A ...	0.75	~1
	49 *	... mG? ...	0.95	1
	68	... CmA,UmA ...	1.82	2
	85	... UmC,UmG	2.22	2
Sum of 18S CH ₃ groups				~45
Previous estimate ^a				~46

The spots itemized are all unusual in that they contain more than one methyl group and/or a methylated base. Two or more methyl groups exist within a single T₁ product in 28S spots 36, 62, 83, 92a and 93 and 18S spots 30, 68 and 85. For four other 28S spots it is not yet clear whether apparent multiple methylation is due to unresolved mixtures of products. Alkali stable dinucleotides or trinucleotides are underlined, for example, UmC signifies 2'-O-methyl uridylyl cytidylic acid. Product X in spot 93 seems to be a triply methylated tetranucleotide, and is identical to product X of ref. 8. Only the mononucleotide of methylated bases is underlined. Identification of the source of these bases is tentative, but a clear distinction can be drawn between base methylated products and ribose methylated products from alkali digestion and subsequent dephosphorylation. Observed molar yields are based on relative amounts of methyl label in three separate T₁ or T₁ and phosphatase fingerprints. A suggested value of 2 signifies two methyl groups, but only 1 mol of the product for a doubly methylated product (for example spot 62).

* Late methylated products.

a fairly high degree of sequence homogeneity within an animal cell rRNA. This has also been inferred from other lines of evidence^{17,18}, and is of particular interest in view of the large number of genes which code for rRNA in animal cells¹⁹.

The presence of a late methylated sequence similar to m₂⁶Am₂⁶ACUG within the smaller ribosomal subunit of two unrelated organisms, yeast¹⁰ and *E. coli*¹¹⁻¹³, suggests that a similar sequence may be of widespread occurrence and may play some general role. Such a role could be connected with the final stages of maturation of the small subunit, as suggested¹¹, or with some function during protein synthesis itself, possibly involving a hydrophobic interaction between the small subunit and one of the other components. Methylation of this sequence, however, does not seem to be absolutely essential, at least in *E. coli*, as a viable mutant exists (resistant to the antibiotic kasugamycin), in which the sequence is not methylated²⁰.

We thank J. Forbes for skilled technical assistance. This work was supported by grants from the Cancer Research Campaign and the Medical Research Council.

Received March 20; revised April 30, 1973.

¹ Greenberg, H., and Penman, S., *J. molec. Biol.*, **21**, 527 (1966).

² Zimmerman, E. F., and Holler, B. W., *J. molec. Biol.*, **23**, 149 (1967).

³ Wagner, E., Penman, S., and Ingram, V., *J. molec. Biol.*, **29**, 371 (1967).

⁴ Weinberg, R. A., and Penman, S., *J. molec. Biol.*, **47**, 169 (1970).

⁵ Vaughan, M. H., Soeiro, R., Warner, J. R., and Darnell, J. E., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1527 (1967).

⁶ Zimmerman, E. F., *Biochemistry*, **7**, 3156 (1968).

⁷ Maden, B. E. H., Salim, M., and Summers, D. F., *Nature new Biol.*, **237**, 5 (1972).

⁸ Maden, B. E. H., Lees, C. D., and Salim, M., *FEBS Lett.*, **28**, 293 (1972).

⁹ Brownlee, G. G., and Sanger, F., *J. molec. Biol.*, **23**, 337 (1967).

¹⁰ Klootwijk, J., Bos, R. C. van den, and Planta, R. J., *FEBS Lett.*, **27**, 102 (1972).

¹¹ Ehresmann, C., Fellner, P., and Ebel, J.-P., *FEBS Lett.*, **13**, 325 (1971).

¹² Hayes, F., Hayes, D., Fellner, P., and Ehresmann, C., *Nature new Biol.*, **232**, 54 (1971).

¹³ Fellner, P., Ehresmann, C., Stiegler, P., and Ebel, J.-P., *Nature new Biol.*, **239**, 1 (1972).

¹⁴ Fellner, P., *Eur. J. Biochem.*, **11**, 12 (1969).

¹⁵ Maden, B. E. H., Salim, M., and Shepherd, J., in *The Structure and Formation of Eukaryotic Ribosomes* (Biochem. Soc. Symposium, 1972, in the press).

¹⁶ Klootwijk, J., Bos, R. C. van den, and Planta, R. J., *Abstr. Commun. Meet. Fed. Eur. Biochem. Soc.*, **8**, No. 492 (1972).

¹⁷ Birnstiel, M. L., Grunstein, M., Spiers, J., and Hennig, W., *Nature*, **223**, 1265 (1969).

¹⁸ Nazar, R. N., and Busch, H., *Biochim. biophys. Acta*, **299**, 428 (1973).

¹⁹ Maden, B. E. H., *Prog. Biophys. molec. Biol.*, **22**, 127 (1971).

²⁰ Helsner, T. L., Davies, J. E., and Dahlberg, J. E., *Nature new Biol.*, **233**, 12 (1971).

Mid-plate Tectonics

D. L. TURCOTTE

Department of Geological Sciences, Cornell University, Ithaca, New York 14850

E. R. OXBURGH

Department of Geology and Mineralogy, University of Oxford

There are many examples of mid-plate tectonics which require an explanation. Oceanic island chains, continental graben and rift valleys, and features which pass from continental areas into oceanic parts of the same plate, are particularly important. We argue that these are the result of crustal extension due to tensional stresses — thermal stresses due to the cooling of the lithosphere, membrane stresses due to changes in the radii of curvature or a complicated combination of both.

THE theory of plate tectonics has explained the location and cause of most seismicity, volcanism and active mountain building¹, all of which are associated with plate margins: ocean ridges where plates are created, ocean trenches where plates descend into the mantle, zones of continental collision, and great faults where lateral motion between plates takes place.

There are, however, several striking exceptions where such tectonic activities occur away from plate margins, for example, the Hawaiian Islands where extensive volcanism has created a mountain range near the centre of one of the largest plates. It also seems that the age of volcanism in the Hawaiian chain increases systematically away from the present volcanism. Such "mid-plate" tectonic phenomena can also occur in continental regions, such as the highly active seismic zone centred near New Madrid, Missouri. This seems to be the termination of a belt of seismicity and tectonic activity which extends from eastern Quebec and is associated with the development of the St Lawrence River Valley².

Hot Spots and Plumes

Wilson³ attributed the island volcanicity of Hawaii to the rise of magmas from a nearly stationary "hot spot" in the upper mantle. The motion of the Pacific plate over the magma source resulted in the formation of the island chain. A modification of this theory has been given by McDougall⁴.

Morgan^{5,6} has further developed the hot spot theory; he identified a large number of apparent hot spots and attributed the magma source to solid state convective plumes rising from the lower mantle. Assuming that the plumes are fixed with respect to the rotational axis of the Earth, he has used the location and age of the island chains to establish the absolute motion of the Pacific plate. The only real test that has been proposed for the hypothesis is a demonstration that the hot spots or plumes are fixed relative to the rotational axis. Morgan⁷ found that this could not be done without allowing the plumes to migrate at velocities between 0.5 and 1 cm yr⁻¹.

McElhinny⁸ has also questioned the validity of the fixed hot spot hypothesis.

Yield and Fracture

An alternative hypothesis for the origin of the Hawaiian chain is a propagating tensional fracture in the lithosphere which causes volcanic activity as it propagates⁹⁻¹¹. The Hawaiian Islands exhibit tensional features; the shield volcanoes seem to be spaced along en-echelon fractures¹² (Fig. 1a). In order to examine this structure in terms of the theory for plastic yielding and fracture of elastic solids we consider the mode of failure of the lithosphere under tension. We assume that brittle fracture will occur near the Earth's surface where the hydrostatic pressure is low but that plastic yielding will occur at depths where the hydrostatic pressure is large compared with the yield stress. The plastic yielding must take place concurrently with the surface fracture to maintain the integrity of the plate. The conditions under which a lithosphere will yield plastically have been derived by Bijlaard¹³ (see also Nadai¹⁴). Assuming that the Mises-Hencky-Huber criterion for yield strength is applicable, Bijlaard showed that a plate under tension would yield plastically at an angle $\beta_f = 35^\circ$ (Fig. 1b).

The near-surface pattern of brittle fracture cannot be predicted theoretically. In continental regions there is evidence that the result of a tensional fracture is a graben structure. Studies of the Rhine graben¹⁵ show that the sunken central block is bounded by normal faults with an inclination of 60° – 65° . This result is in agreement with the Coulomb-Navier theory for fracture if the coefficient of internal friction is taken to be 0.58 to 0.84.

Clearly the mode of tensional fracture in ocean areas differs from that in continental areas. There is little evidence of graben structures in the Hawaiian Islands. Instead the shield volcanoes seem to lie along en-echelon fractures which have opened and have been filled with magma. The difference may be due to a greater availability of magmas from the asthenosphere in ocean areas. We assume that the oceanic lithosphere fractures under tension in the direction of maximum shear stress, at an angle $\beta_f = 45^\circ$ (Fig. 1b). In terms of the Coulomb-Navier theory this corresponds to zero internal friction. When finite deformation takes place these fractures open and fill with magma. The resulting pattern of brittle fractures inclined at 45° and a zone of plastic flow inclined at 35° is similar to the structure of the Hawaiian Islands (Fig. 1a). Because the principal trend of the Hawaiian Islands is at 22° to lines of latitude, the predicted direction of the tensional stress is 13° to the west of north and 13° to the east of south.

It is not easy to see how a mantle hot spot or source of magma would result in regular tensional fractures. Localized heating would cause thermal expansion of the lithosphere which would lead to compression. It is possible that shear stresses associated with a plume could operate on the base of the lithosphere but it is not clear how they could produce the observed features. What are the alternative sources for the

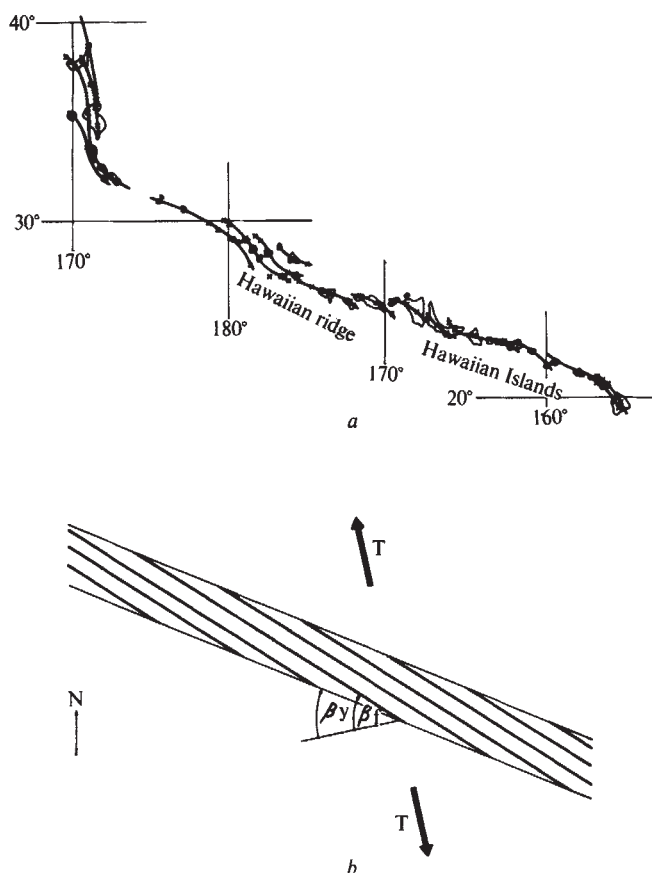


Fig. 1 *a*, Structure of the Hawaiian Islands after Jackson *et al.*¹². Apparent shield volcanoes (x) seem to lie along en-echelon fractures (—). *b*, Predicted pattern of plastic yielding at an angle $\beta_y = 35^\circ$ and brittle fractures at an angle $\beta_f = 45^\circ$ due to a tensional stress shown by the vectors *T*.

tensional stresses that seem to be responsible for the Hawaiian Islands?

Thermal Stresses

The lithosphere is created in the vicinity of an ocean ridge by the cooling of mantle rock. As the lithosphere convects away from the ridge further cooling takes place and its thickness increases. The lower boundary of the lithosphere can be defined as the isotherm T_y ; at temperatures less than T_y there are no significant deformations of the mantle rock on geological time scales at stresses below the yield stress.

An elastic plate which is cooled non-uniformly is subject to thermal stresses. We consider a lithosphere which is

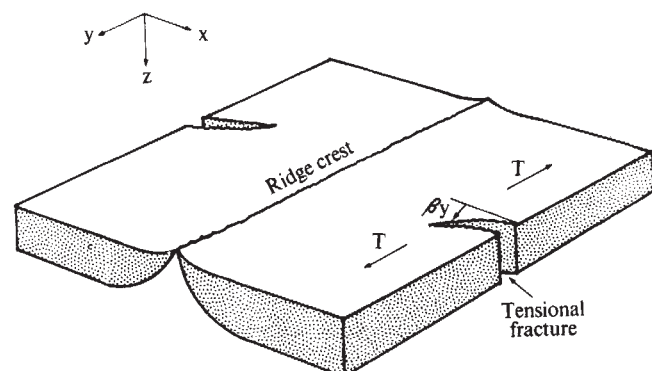


Fig. 2 Illustration of fractures due to thermal stresses in spreading plates. Tensions indicated by vectors *T*.

cooling as it moves away from an ocean ridge. We take the direction parallel to the ridge axis to be the *y* direction, the direction perpendicular to the ridge axis to be the *x* direction, and the normal to the surface to be the *z* direction as shown in Fig. 2. We assume that deformation in the *y* direction is zero so that the thermal stress is given by¹⁶

$$(\sigma_{yy})_{th} = \alpha E (T_y - T_0) \quad (1)$$

where α is the coefficient of thermal expansion, E is Young's modulus, and T_0 is the temperature to which the plate is cooled. The resultant stress is a tension parallel to the ridge

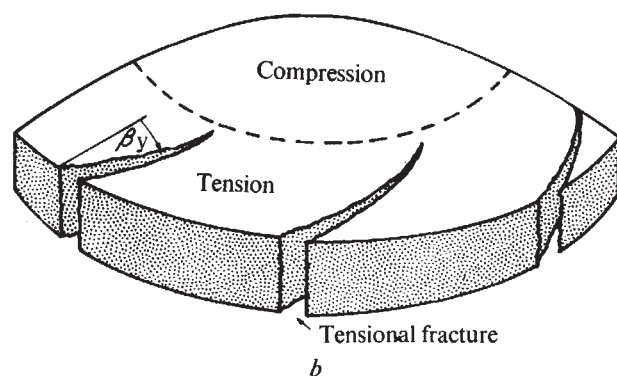
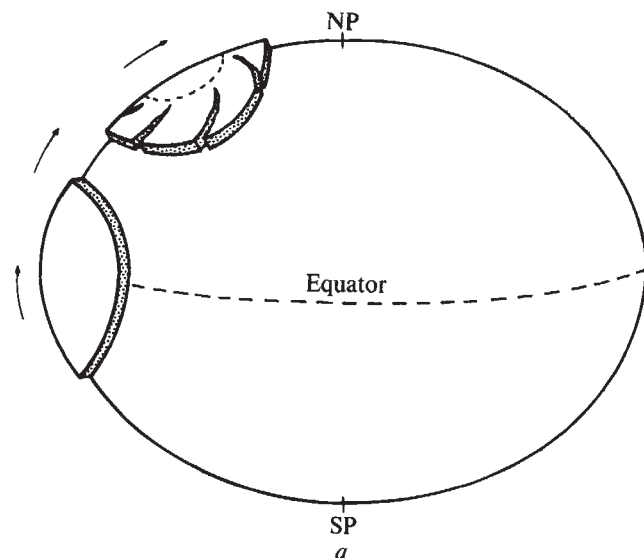


Fig. 3 Illustration of fractures due to non-spherical plate tectonics. *a*, Movement of a plate northwards from the equator showing edge fractures due to an increase in radii of curvature. *b*, Distribution of stresses and fractures due to an increase in radii of curvature in a circular plate.

axis. Taking $\alpha = 3 \times 10^{-5} \text{ } ^\circ\text{C}^{-1}$, $E = 1.7 \times 10^{12} \text{ dyne cm}^{-2}$, $T_y = 800^\circ \text{C}$, and $T_0 = 0^\circ \text{C}$ as typical properties for a lithosphere we find that $(\sigma_{yy})_{th} = 4 \times 10^{10} \text{ dyne cm}^{-2}$. This thermal stress is sufficient to fracture the lithosphere.

Thermal stresses due to the cooling of an oceanic lithosphere will be tensions parallel to the ridge where the plate was created. Therefore, the stresses will be aligned with the magnetic anomalies in the ocean floor and will be normal to fracture zones. From the trends of the Murray and Molokai fracture zones and adjacent magnetic anomalies, the direction of the tensional thermal stresses near Hawaii should be 14° west of north and east of south. This is within one degree of the direction predicted in the previous section from the trend of the island chain.

Non-spherical Plate Tectonics

Tensional stresses in a lithosphere may also be caused by membrane stresses due to non-spherical plate tectonics. If the Earth was a perfect sphere the spherical surface plates would be free to move about without deformation. But to a good approximation the Earth is an oblate spheroid with an ellipticity of 0.00335. The principal radii of curvature at the equator are 6,378 and 6,335 km; at the poles the radii of curvature are equal, 6,400 km. If a lithosphere is created on one part of the spheroid it must deform when its latitude changes, for its radii of curvature must change. A plate which is created in the vicinity of the equator must have its radii of curvature increased if the latitude of the plate changes (Fig. 3a).

Bending moments are associated with changes in radii of curvature. But for a shell the thickness of which is small compared with its radii of curvature, the stresses associated with bending are small compared with the membrane stresses in the shell. The membrane stresses are simply the result of stretching the plate to different radii of curvature.

According to membrane theory¹⁷ the magnitude of the stresses required to stretch a segment of a shell from one radius of curvature to a slightly different radius of curvature is given by

$$\sigma_M = C_1 E \Delta R / R \quad (2)$$

where R is the initial radius of curvature, ΔR is the change of radius of curvature, and C_1 is a constant of order 1. If the radius of curvature of a circular segment of a spherical shell is increased, the edge of the shell will experience a tangential tensional stress and the centre of the shell will be in compression (Fig. 3b).

Assuming that ΔR is the difference between the radii of curvature at two locations on the Earth the ratio $\Delta R/R$ is related to the ellipticity of the Earth ε by

$$\Delta R/R = C_2 \varepsilon \quad (3)$$

where C_2 is a function of the difference in latitude between the two points on the Earth. If this difference is a significant fraction of 90° then the constant C_2 will be of order 1. Combining equations (2) and (3) gives

$$\sigma_M = C_1 C_2 E \varepsilon \quad (4)$$

With $\varepsilon = 0.00335$, $E = 1.7 \times 10^{12}$ dyne cm⁻², and setting $C_1 C_2 = 1$ we find that $\sigma_M = 5.7 \times 10^9$ dyne cm⁻². The membrane stresses due to the movement of the lithosphere over the surface of the non-spherical Earth are sufficient to fracture the lithosphere.

Fractures due to membrane stresses would be expected to

appear in mid-latitudes because the change in the radii of curvature of the Earth with latitude is a maximum at a latitude of 45°. The point of generation of such fractures would be nearly fixed in latitude. For northward moving plates in the northern hemisphere such as the Pacific plate and the Americas plate the crack should be propagating southwards. This is the case for both the Hawaiian Islands and the New Madrid, Missouri-St Lawrence Valley tectonic zone.

Non-spherical plate tectonics requires a finite extension to relieve the membrane stresses. This is observed in rift valleys and graben structures. The Rhine graben has a mean width of 36 km and the extension across the valley is estimated to be 4.8 km (ref. 15). Other rift and graben structures have widths and crustal extensions of the same order. Burke *et al.*¹⁸ associate the Rhine graben with a hot spot. It is, however, not clear how a hot spot or plume could lead to the known unidirectional crustal extension across the Rhine Valley. Non-spherical plate tectonics provides an explanation for a permanent extension of the crust of this magnitude.

There may be further implications of both thermal stresses and membrane stresses. For example, the fractures caused by these stresses may initiate the breakup of continents, for example, for the formation of the Atlantic Ocean. Also, the additional force required to change the latitude of a plate due to the membrane stresses would favour east-west seafloor spreading over north-south seafloor spreading.

Received March 20, 1973.

- ¹ Isacks, B., Oliver, J., and Sykes, L. R., *J. geophys. Res.*, **73**, 5855 (1968).
- ² Kumarapeli, P. S., and Saull, V. A., *Can. J. Earth Sci.*, **3**, 639 (1966).
- ³ Wilson, J. T., *Can. J. Phys.*, **41**, 863 (1963).
- ⁴ McDougall, L., *Nature phys. Sci.*, **231**, 141 (1971).
- ⁵ Morgan, W. J., *Nature*, **230**, 42 (1971).
- ⁶ Morgan, W. J., *Bull. Am. Ass. Petrol. Geol.*, **56**, 203 (1972).
- ⁷ Morgan, W. J., *Geol. Soc. Am. Mem.*, **132** (in the press).
- ⁸ McElhinny, M. W., *Nature*, **241**, 523 (1973).
- ⁹ Betz, F., and Hess, H. H., *Geogr. Rev.*, **32**, 99 (1942).
- ¹⁰ Jackson, E. D., and Wright, T. L., *J. Petrol.*, **11**, 405 (1970).
- ¹¹ Green, D. H., *Phil. Trans. R. Soc.*, **268**, A, 707 (1971).
- ¹² Jackson, E. D., Silver, E. A., and Dalrymple, G. B., *Bull. geol. Soc. Am.*, **83**, 601 (1972).
- ¹³ Bijlaard, P. P., *Ingenieur in Nederl. Indie*, **11**, 135 (1935).
- ¹⁴ Nadai, A., *Theory of Flow and Fracture of Solids*, **1**, second ed., 316 (McGraw-Hill, New York, 1950).
- ¹⁵ Illies, J. H., in *Graben Problems* (edit. by Illies, J. H., and Mueller, St.), **4** (Schweizerbart'sche Verlagsbuch, Stuttgart, 1970).
- ¹⁶ Boley, B. A., and Weiner, J. H., *Theory of Thermal Stresses*, 279 (Wiley, New York, 1960).
- ¹⁷ Novozhilov, V. V., *The Theory of Thin Shells*, 102 (Noordhoff, Groningen, 1959).
- ¹⁸ Burke, K., Kidd, W. S. F., and Wilson, J. T., *Nature phys. Sci.*, **241**, 128 (1973).

LETTERS TO NATURE

PHYSICAL SCIENCES

Subsidence of the Venice Area during the Past 40,000 yr

THE present day subsidence of the Quaternary deposits of the plain of Venice can be considered to be the result of either geological factors—the plain is situated on the northern margin of the subsident area of the Po valley—or of human activity over several centuries, such as overexploitation in the confined aquifers of low turnover¹, urban and industrial development on unconsolidated sediments.

The respective contribution of the different factors affecting subsidence may vary from place to place. Drillings, continuous corings^{2,3} and seismic profiles⁴ can provide average sedimentation rates of about 0.5 to 1.0 mm yr⁻¹ since the Upper Pliocene from north to south of the lagoon of Venice. Geodesic studies⁵, available since the early 1900s, have shown that in some places, Lido di Venezia for instance, the soil is now sinking at a velocity close to 8 mm yr⁻¹.

In order to study the geological component of subsidence at an intermediary time scale, systematic analysis of the chronology of the upper Quaternary deposits has been undertaken using ¹⁴C measurements. This study is designed to determine whether or not "geological" subsidence for the past 40,000 yr

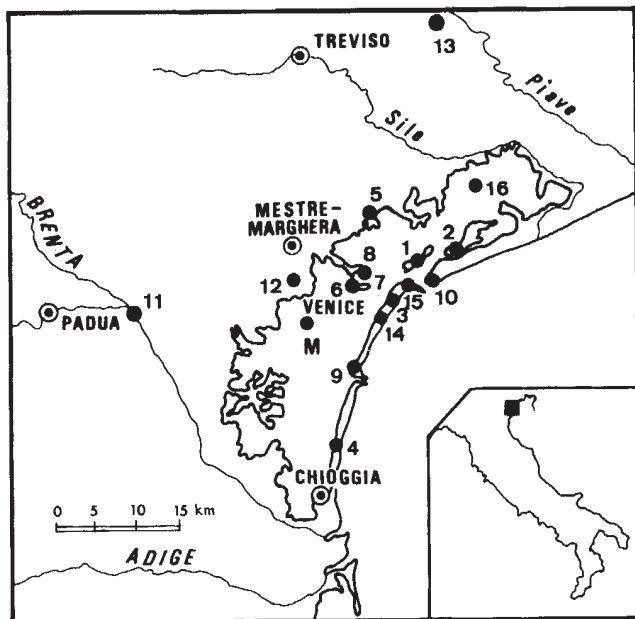


Fig. 1 Location of samples. 1, Sant' Erasmo; 2, Treporti; 3, Lido of Venice San Nicolo; 4, Lido di Pellestrina; 5, Cà Montiron; 6, Venice Drilling CNR I; 7, Venice civil hospital; 8, Venice civil arsenal; 9, Alberoni; 10, Punta Sabbioni; 11, Alveo Brenta; 12, Conca Romea; 13, Candelù; 14 and 15, Lido of Venice; 16, Canale Cenosa; M, Motte di Volpego^{9,14,16}.

has been constant. If the subsidence has varied, has there been an acceleration in recent times?

The samples studied consisted of peats and peaty muds, vegetal relicts, shells, and carbonates. All of the samples were

collected as cores whose locations are given in Fig. 1. The ^{14}C measurements were performed by liquid scintillation after synthesis of benzene⁹. No correction was applied to the apparent ages.

Peats located throughout the area give a regular pattern when we plot depth against present soil surface (Fig. 2a). The correlation is somewhat better if related to the average sea level (Fig. 2b), except for the samples from inland (samples 11 and 13). From about 40,000 to 22,000 yr BP the correlation between age and depth is linear. The slope is about 1.2 mm yr^{-1} . Between 22,000 and 18,000 yr BP a very high sedimentation rate produced at least 17 m of deposits. The basin has not given any more continental document since 18,000 yr BP, but the total thickness of this deposit may have been greater because it is terminated by an unconformity.

The information derived from the marine shells is very different. None is older than 5,900 yr, although they are at times located at depths greater than peats which laterally are much older. The shelly deposits can reach more than 20 m for the past 5,900 yr. In a plot of depth against age there is no general correlation. Further, measurements on shells from a single core do not show linear age-depth relations but give rough curves showing that the rate of marine deposition has decreased between 5,900 and 2,020 yr BP. The accumulation rate is greater than that which could be attributed to the Flandrian transgression (Fig. 2b).

The radiometric results lead to the following sequence of geological events.

(1) Continental sedimentation. Peats and interbedded fine-grained terrigenous sediments are thought to represent repeated fluvio-lacustrine cycles. The regularity of the apparent ages given by peats coming from different locations which are widely spaced suggests that sedimentation took place in a basin isolated from the sea at a constant rate of accumulation which

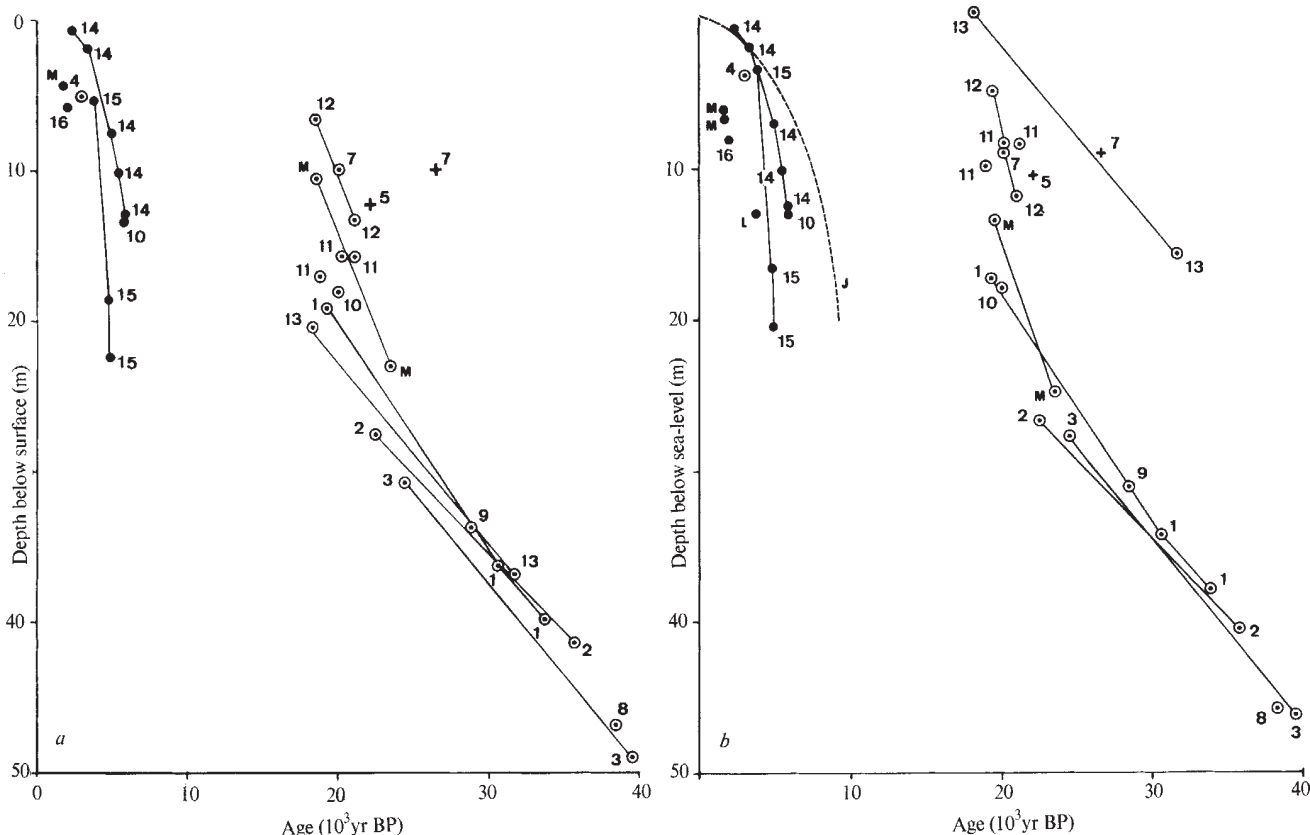


Fig. 2 Age/depth relation. *a*, Relative to ground level; *b*, relative to present sea level. Locations as Fig. 1. ○, Peats; ●, marine shells; +, limy fraction of the sediment suspected of containing reworked carbonate. Peat of location 4 (Lido di Pellestrina) must be related to a stage of Flandrian transgression. We note that for continental deposits the correlation between age and depth is generally better if plotted using present sea level except for the samples coming from the inland (Candelù; location 13). During the Holocene, the accumulation rate is greater than the general Flandrian transgression rate as indicated by curve J (ref. 13) for the Netherlands. Previous radiometric data were obtained on core at Motte di Volpego¹⁴ (M) and on the immersed beach rock at Lignano, 80 km NE of Venice¹⁶ (L).

was essentially controlled by the subsidence for the interval 40,000 to 22,000 yr BP. The average rate (1.2 mm yr^{-1}) seems greater than that (0.5 to 1.0 mm yr^{-1}) determined by seismic methods in the Venice area⁴. The deposits remain continental in character between 40,000 and 30,000 yr BP which is an epoch of relatively high sea level in the eastern Mediterranean⁷. The basin was not invaded and, assuming a minimum value of 1.2 mm yr^{-1} for subsidence, it seems that the basin was at least 10 to 15 m above sea level at that time.

The drastic increase in sedimentation from 22,000 to 18,000 yr BP can be attributed to an increase of water supply (and sediments) due to an increase in the pluviosity and to a sudden increase in local or regional continental subsidence.

This period of 22,000 to 18,000 yr BP covers the glaciation maximum which is generally correlated with a pluvial epoch. But in this area the fluvial supply is considered low because of the water retention in the Alpine glaciers⁹ as indicated further by palynological data which suggest a rather dry climate⁹ in the area.

Although the development of the alpine glaciers during the last Würmian episode was probably very extensive¹⁰, we cannot demonstrate any epirogenic effect which would have effected an overcharge of ice on the continent, but a tectonic effect is highly probable in the next period (between 18,000 and 5,900 yr BP). It could have begun at about 22,000 yr BP.

Whatever the reason, the accumulation of sediment during this period was at least 5 mm yr^{-1} .

(2) Continental emersion. Between the last continental deposits dated in the area and the earliest marine accumulation recorded in the cores, there is an interval of about 12,000 yr (approximately 18,000 to 6,000 yr BP). Because of the possible effect of post-depositional erosion during this period, the value for the break is a maximum. During this time the balance deposition-erosion was equal to zero or negative. It probably occurs as a general decrease of the level of the aquifers correlated with the epoch of lowest sea level¹¹. The shallower argillaceous deposits of the previous episode are compacted and oxidized¹² and give an indurated level, the so-called "caranto", the most stable layer for foundation works in the area. This level is representative of the limit Pleistocene-Holocene.

The results from Candelù suggest that the whole area was tilted by an upward movement on the continent and probably a downward movement toward the Adriatic basin. This movement is indicated by the shift of the representative points (No. 13) if we plot apparent age against present sea level instead of against group surface (Fig. 2b).

(3) Marine transgression. Several factors (variations in initial ^{14}C content of dissolved carbon in brackish ambients, accumulation of organisms either as biocoenosis or as thanato-coenosis, variable thickness of water at the moment of deposition) can lead to non-consistent values in the relationship between radiometric ages and sea levels. Nevertheless, certain statements can be made. The sea came into the basin at about 6,000 yr BP with the Flandrian transgression. It was the first marine incursion in the basin since before 40,000 yr BP and probably since, at least, the neothyrrhenian (Ouljian) transgression at about 80,000 yr BP (ref. 11). The Flandrian transgression reached a maximum after 2,000 yr BP as shown by the age at the upper part of the Lido island in the lagoon. The sea covered a relief of deep channels (at least 20 m in the north of Lido island, location 15 on Fig. 1) of tidal origin or those individualized earlier by river erosion. The deposition of shells then took place in these channels very rapidly at the beginning of the transgression and then more slowly. The poor correspondence for the combined data between age and depth for the shell material is probably due to the control of their accumulation rate by the size and biotic activity in the respective channels. All the representative points in the age/depth diagram lie under the curve established for the Netherlands¹³. This means that the accumulation rate of marine deposits was greater than the rate of Flandrian transgression. Hence the subsidence was still going on, although attenuating because the upper points at

about 3,000 yr BP are very close to the general curve. So the beginning of the human settlement in the area seems to coincide with a period of ground stability.

These conclusions are in good agreement with the few data already obtained on the vertical profile in Motte di Volpego^{9,14,15}. But the concept of a continuous fluvial and lacustrine sedimentation, from about 25,000 yr BP to 2,000 yr BP (Fig. 24 in ref. 14), is no longer compatible with the results obtained.

The palaeogeographical evolution of the area can be summarized as follows: continuous subsidence in a marshy continental basin with approximately the same rate of accumulation for sediments and peats (from 40,000 yr BP and before, until 22,000 yr BP, this rate is about 1.2 mm yr^{-1}); drastic increase in the same kind of accumulation up to 5 mm yr^{-1} and maybe more in the same basin between 22,000 yr BP and at least 18,000 yr BP as a very probable consequence of tectonics (emersion stage with induration of the superficial levels at the end of the episode); marine transgression since about 6,000 yr BP in channels of erosion, and then in the whole area, accompanied by high rate of accumulation.

This work was sponsored by the Centre National de la Recherche Scientifique and the Consiglio Nazionale delle Ricerche. Most of the samples were provided by Professor P. Colombo and Dr C. Rizzetto. Professors R. Frassetto and R. Malaroda, Drs E. Mutti and B. Velde offered helpful comments.

J. CH. FONTES

Laboratoire de Géologie Dynamique,
4, place Jussieu, Paris V°

G. BORTOLAMI

Laboratorio per lo Studio della Dinamica delle Grandi Masse,
Cà Papadopoli 1364, San Polo, Venice

Received May 23, 1973.

¹ Bortolami, G., Fontes, J. Ch., and Panichi, C., *Earth Planet. Sci. Lett.* (in the press).

² ENI, *Acque Dolci Sotteranee*, XXIII, 914 (1972).

³ CNR, *CNR Laboratorio per lo Studio della Dinamica delle Grandi Masse, Venezia, tech. Rep.*, 15 to 21 (1971).

⁴ Finetti, I., and Morelli, C., *Boll. geof. Teor. Appl.*, XIII 44 (1972).

⁵ Caputo, M., Folloni, G., Gubellini, A., Pieri, L., and Unguendoli, M., *CNR Laboratorio per lo Studio della Dinamica delle Grandi Masse, Venezia, tech. Rep.*, 9 (1971).

⁶ Fontes, J. Ch., *Rev. Géog. Phys. Géol. Dyn.*, XIII, 67 (1971).

⁷ Fontes, J. Ch., and Perthuisot, *Rev. Géog. Phys. Géol. Dyn.*, XIII, 299 (1971).

⁸ Mosetti, F., and d'Ambrosi, C., *Atti. Mem. Commiss. Grotte "E. Boegan"*, VI, 19 (1966-67).

⁹ Bertolani Marchetti, D., *Mem. Biogeogr. Adriat.*, 7, 53 (1966-67).

¹⁰ *Suppl. Bull. Assoc. Fr. Et. Quat.* (Paris, 1969).

¹¹ Guilcher, A., *Earth Sci. Rev.*, 5, 69 (1969).

¹² Matteotti, G., *Notiziario Ordine Ing. Prov. Padova.*, 6, 12 (1962).

¹³ Jelgersma, S., *Medel. Geol. Sticht.*, C, 6 (1961).

¹⁴ Bonatti, E., *Mem. Biogeogr. Adriat.*, suppl., 7, 55 (1968).

¹⁵ Ascoli, P., *Mem. Biogeogr. Adriat.*, 7, 53 (1966-67).

¹⁶ Stefanon, A., *Mem. Biogeogr. Adriat.*, 8, 79 (1969-1970).

Evolution of Triple Junctions

THE boundaries between the rigid plates which make up the Earth's crust are recognized as being of three distinct kinds: ridges, from which new crustal material spreads symmetrically; trenches, at which plates descend back into the mantle; transform faults, along which plates may slip relative to each other¹⁻³. An important concept is the triple junction⁴, which is the point at which three plate boundaries meet. Discussions of the development of oceanic and continental crustal features almost inevitably contain considerations of motion, stability and instability of triple junctions. McKenzie and Morgan⁵ showed there are sixteen possible triple junctions and set out rules for examining their stability. They concluded that fourteen of them were stable under certain conditions. Here I point out that fifteen of the triple junctions are stable in the appropriate conditions.

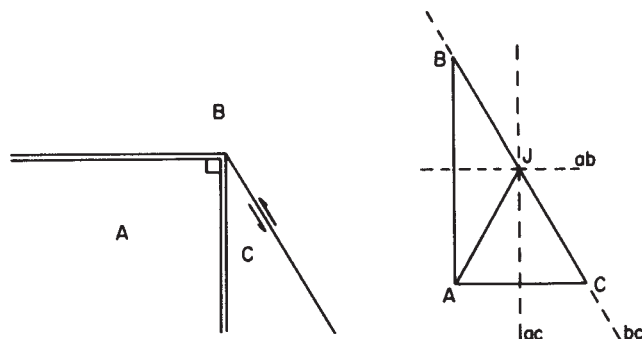


Fig. 1 Example of a stable RRF triple junction along with its velocity triangle. The two ridges meet at right angles. J is the representative point of the triple junction.

In what follows R, T and F stand for ridge, trench and fault, respectively. I assume that at a ridge, spreading occurs at right angles to the strike of the ridge, and that lithosphere is consumed only from one side at a trench.

I consider the ridge-ridge-fault triple junction (RRF) which McKenzie and Morgan stated is unstable. I will show that such junctions can be constructed which are stable if the two ridge elements meet at right angles as in Fig. 1. The condition that the three plates A, B and C be in rigid body relative motion is that the relative velocity vectors for the plates should form a triangle. In Fig. 1 I show the appropriate velocity triangle. As McKenzie and Morgan pointed out, there are, however, additional conditions required for triple junction stability. That is, one must find, in the velocity space in which the triangle is drawn, a point representing the relative velocity of a reference frame in which the triple junction is stationary. If such a point exists, the triple junction is stable. This point is found in Fig. 1, using the procedure of McKenzie and Morgan. Thus an observer moving with uniform velocity along ridge AB (the ridge dividing A from B) would have his motion represented by a point on the perpendicular bisector (ab) of side AB of the triangle. If this observer were actually fixed relative to the ridge, his representative point would be at the midpoint of AB. Similarly, an observer moving along ridge AC with uniform velocity would have a representative point in velocity space on the perpendicular bisector (ac) of the side AC. An observer moving along the transform fault CB with uniform velocity would have a representative point somewhere on bc which is a straight line encompassing side BC and its extensions in both directions. Now these three lines, ab, ac and bc, meet at a point (Fig. 1), so an observer moving with a velocity represented by this intersection point would be able to remain above both ridges and the fault simultaneously, which is only possible at the triple junction. Therefore, there does exist a reference frame in which the triple junction is stationary and the RRF arrangement of Fig. 1 is stable, which completes the proof. The velocity of this particular reference frame with respect to plate A is clearly given in magnitude and direction by the line AJ in Fig. 1.

I note that only when the two ridge elements are orthogonal is the RRF triple junction stable. This is because only then do the two perpendicular bisectors ab and ac intersect on bc. The particular case drawn by McKenzie and Morgan has the two ridges forming a continuous straight line with the fault lying perpendicular to this line. This is an unstable junction, as they note, but as I have just shown, their general conclusion that all RRF triple junctions are unstable is not correct.

Finally, I point out that the point J in velocity space giving the velocity with which the ridge-ridge-ridge (RRR) triple junction moves is the circumcentre of the appropriate velocity triangle and not in general the centroid as stated in the original paper⁶; and the stability condition for the

ridge-ridge-trench (RRT) triple junction given by McKenzie and Morgan should be changed to read "ab must go through the circumcentre of ABC".

I thank Professor G. D. Garland for discussions.

DEREK YORK

Geophysics Division,
Department of Physics,
University of Toronto, Toronto

Received May 9, 1973.

¹ McKenzie, D. P., and Parker, R. L., *Nature*, **216**, 1276 (1967).

² Morgan, W. J., *J. geophys. Res.*, **73**, 1959 (1968).

³ Le Pichon, X., *J. geophys. Res.*, **73**, 3661 (1968).

⁴ Isacks, B., Oliver, J., and Sykes, L. R., *J. geophys. Res.*, **73**, 5855 (1968).

⁵ Wilson, J. T., *Nature*, **207**, 343 (1965).

⁶ McKenzie, D. P., and Morgan, W. J., *Nature*, **224**, 125 (1969).

Latitudinal Extent of Late Precambrian Glaciations

LATE Precambrian glaciations are widely recognized to have been extensively distributed (see, for example, ref. 1) but there are few quantitative data to show whether they reached low latitudes. If this can be demonstrated it will be legitimate to regard Late Precambrian tillites as stratigraphic marker horizons².

Geochronological studies of recent years have given some absolute control of the age of many of the tillite occurrences. According to Dunn *et al.*³ the Egan-Morinoan Glaciation of Australia is 670 m.y. old and the Moonlight Valley-Sturtian Glaciation is 750 m.y. old. A time span of 675–560 m.y. is given³ for the Vendian of the USSR, and Pringle⁴ concludes that the Varanger Ice Age of northern Norway occurred at about 668 ± 23 m.y. Stratigraphically controlled ages define the intervals of late Precambrian glaciation in Africa. The Grand Conglomérat of Zaïre was deposited both as a tillite and a fluvio-glacial deposit and has a maximum probable age of 950 m.y. (ref. 5). The Petit Conglomérat was deposited between two early phases of the Katangan orogeny dated at 710 m.y. and 670 m.y. BP (ref. 5). Schemerhorn and Stainton⁶ equate the Lower and Upper "tilloids" of the West Congo System in west-central Africa with the Grand and Petit Conglomérats. Tillites of probable similar age are found in Ghana, Togo, Dahomey, the western Sahara and the Anti-Atlas of Morocco, and a possible late Precambrian tillite is found in Sierra Leone and Guinea⁷.

In the Orange River area of south-western Africa, Kröner⁸ found cold water rudites and signs of fluvio-glacial sedimentation in the Upper Hilda Formation of the Gariep Group. These may correlate with the Bushmannslippe glacial sediments overlying the Nosib Formation further north and have a maximum age of 719 ± 28 m.y. Also the lower part of the Gariep Group deposited at <850 m.y. indicates a wet and cold climate during deposition. These tillites predate those found in the Nama Group and the Damara Group (with which the Nama is now correlated⁹). The Nama Group has a maximum age of 653 ± 70 m.y. and the topmost member is probably Lower Cambrian. These younger Infra-Cambrian glacial horizons occur in close association with sediments seemingly deposited under warm conditions in low latitudes. The Portaskaig Boulder Bed of the Scottish Dalradian, interpreted as a subaqueous tillite⁹, occurs with limestones, dolomites, algal stromatolites and possible sun-cracked quartzites¹⁰ in a similar setting. A sparse amount of palaeomagnetic data from rocks associated with the glacial deposits of Spitzbergen and Southern Norway suggests that they lay in low latitudes during formation.

A widespread extent for Late Precambrian glaciations has been disputed from analogies with Phanerozoic situations¹¹ and scepticism about the nature of some tillites^{12,13}. Most of the evidence, however, leave no doubt that most of the described formations are tillites⁷, and sufficient palaeomagnetic data are now available to examine the palaeolatitudinal distribution of many occurrences. The poles from Africa spanning the interval during which the Late Precambrian glaciations took place are listed in Table 1, and the north poles are plotted on a stereographic projection in Fig. 1. Africa lay in low latitudes from before 1,000 m.y. until post-Lower Cambrian times, and all results obtained from Africa for the interval 740 m.y. to the Lower Cambrian give pole positions close to the present pole showing that Africa occupied a position close to its present one with respect to the poles for a "quasi-static" interval occupying this period. Analysis of palaeosecular variation²⁰ and the agreement of palaeomagnetic results over large areas of Precambrian shield^{21,22} imply that the geocentric dipole model for the behaviour of the Earth's magnetic field is applicable to at least the latter part of Precambrian time. It is therefore legitimate to draw conclusions concerning the geographic poles from the palaeomagnetic results, and the available data furthermore allow Africa to be considered as an integral continent before the "Pan African" orogeny (600 ± 150 m.y., ref. 14 and my unpublished work with J. C. Briden and K. Lomax).

Localities of Late Precambrian tillites occur throughout the length of Africa⁷ and from the palaeomagnetic data I conclude that they straddle the palaeoequator of the time. The tillite localities of Australia also appear to have lain within 30° of the equator of the time, but it is impossible to be certain of this from the available palaeomagnetic data. The older Precambrian palaeomagnetic poles from Australian haematite ores²³ and dated igneous rocks²¹ suggest that a reconstruction similar to that of Carey²⁴ was applicable to the Proterozoic, although two new Late Precambrian or Lower Cambrian results from Australia²⁵ do not agree with the African results using this reconstruction. Within Africa a very large polar shift in excess of 90° is identified in the Lower Cambrian from the difference between poles 7 to 12 in Fig. 1 and poles 15 to 18. This is the largest single polar shift yet identified in geologic time but is confirmed by South American data²⁶ after correction for Phanerozoic drift (Fig. 1) and may include poles 13 and 14 (Table 1) from Africa. This polar shift does not affect the conclusion that the Late

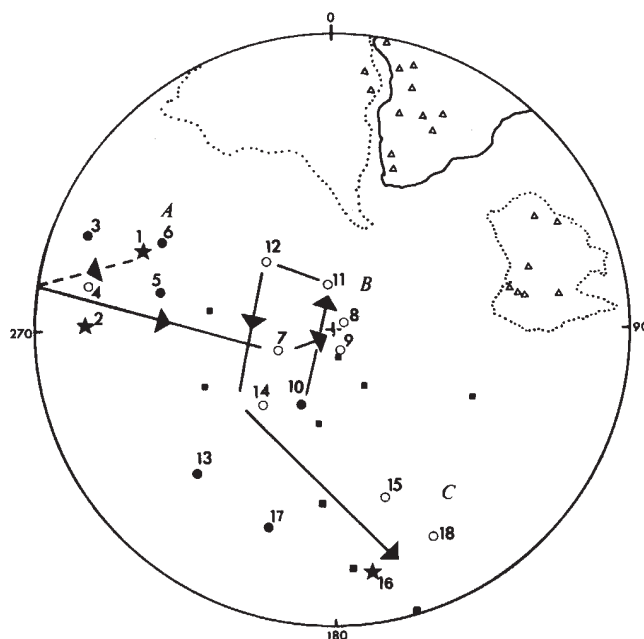


Fig. 1 Palaeomagnetic north poles from Africa for the interval 900 to 500 m.y. plotted on a stereographic projection. The poles are listed in Table 1; closed symbols refer to poles, open symbols to antipoles and stars to pole on the back hemisphere of the projection. The arrowed full line shows the probable migration path of the north pole relative to Africa, the squares are poles of Thompson²⁶ attributed to the Cambrian after correction for Phanerozoic drift. The dotted outline of Australia is the pre-Mesozoic reconstructed position after Cary's²⁴ reconstruction and the triangular symbols refer to Late Precambrian tillite occurrences listed by Harland¹. A, Mostly pre-glacial poles; B, poles for glacial interval; C, post-glacial.

Precambrian tillites of Africa were deposited in low latitudes because it postdates the age of these occurrences. The Fish River Formation pole (No. 12) at the start of the shift lies above the tillite horizons of the Nama Group and is probably basal Cambrian in age²⁷.

Unfortunately the Late Precambrian palaeomagnetic data from North America are sparse, but the apparent polar wander curve of Irving and Park²² places North America entirely between palaeolatitudes of $\pm 20^\circ$ during the interval 800–600 m.y. BP and implies that the North American Late Precambrian tillites were also formed in low latitudes.

Table 1 Palaeomagnetic Poles from Africa Belonging to the Interval 900 to 500 m.y.

Pole No.	Rock unit and locality	Age (m.y.)	Pole position		Reference
			°E	°N	
1	Dykes, Klein Karas, South West Africa	878 ± 41	294	+20	14
2	Kigonero Flags, Tanzania	> 890	273	+11	15
3	Malagarasi Sandstone, Tanzania		292	-7	15
4	Bukoban intrusives, Tanzania	806 ± 30	101	+11	15
5	Gagwe Amygdaloidal Lavas, Tanzania	813 ± 30	283	-29	15
6	Manyovu Red Beds, Tanzania	$< 813 \pm 30$	298	-24	15
7	Mbozi Complex, Tanzania	743 ± 30	68	+72	14
8	Pre-Nama dykes, South West Africa	653 ± 70	228	+85	14
9	Sabaloka Ring Structure, Sudan	> 540	339	+83	16
10	Plateau Series, Zambia (i)		205	-60	14
11	Plateau Series, Zambia (ii)		173	+71	14
12	Fish River Formation, Nama Group, South West Africa	Lower Cambrian	137	+53	14
13	Doornpoort Formation, South West Africa	Remagnetized by Katangan folding (580 to 680 m.y.)	225	-22	14
14	Lavas, Morocco	"Middle Cambrian"	34	+53	19
15	Ntonya Ring Structure, Malawi	630 ± 24	345	+28	17
16	Plateau Series, Zambia (iii)	Lower Palaeozoic	172	+10	14
17	Plateau Series, Zambia (iv)	Lower Palaeozoic	199	-21	14
18	Hook intrusives, Zambia	500 ± 17	336	+14	18

Poles are listed in probable order of age. See references for discussion of quality and age control of the pole positions.

An impressive relationship has been demonstrated in Africa for the Palaeozoic between the incidence of continental glaciation and polar movements. Lower Palaeozoic poles²⁸ agree with the evidence of continental glaciation in north west Africa during Ordovician and early Silurian times, and the shift of the south pole to its Upper Palaeozoic position near southern Africa correlates with the evidence for glaciation here²⁹. This incidence of glaciation with polar movement is not observed in the Late Precambrian, and I conclude that there was a large contraction in the scale and areal extent of glaciation close to the base of the Cambrian. Possible implications of this phenomenon have been discussed elsewhere³⁰.

I thank Professor L. Cahen and Dr A. Kröner for correspondence and Dr J. C. Briden for commenting on the manuscript.

J. D. A. PIPER

Department of Earth Sciences,
University of Leeds,
Leeds LS2 9JT

Received May 3, 1973.

- ¹ Harland, W. B., *Geol. Rdsch.*, **54**, 45 (1964).
- ² Dunn, P. R., Thompson, B. P., and Rankama, K., *Nature*, **231**, 498 (1971).
- ³ Keller, B. M., Korolev, B. P., Semikhatov, M. A., and Chumakov, N. M., in *Geologi ya Dokembriya* (Nauka, Leningrad, 1968).
- ⁴ Pringle, I. R., *Geol. Mag.*, **109**, 465 (1973).
- ⁵ Cahen, L., in *African Magmatism and Tectonics*, 97 (Oliver and Boyd, Edinburgh, 1970).
- ⁶ Schermerhorn, L. J. G., and Stainton, W. I., *Q. Jl geol. Soc. Lond.*, **119**, 201 (1963).
- ⁷ Harland, W. B., in *Problems in Palaeoclimatology*, 119 (Interscience, London, 1963).
- ⁸ Kröner, A., *Geol. Rdsch.*, **60**, 1515 (1971).
- ⁹ Kilburn, C., Pitcher, W. S., and Shackleton, R. M., *Geol. J.*, **4** (2), 343 (1965).
- ¹⁰ Johnson, M. R. L., in *The Geology of Scotland*, 115 (Oliver and Boyd, Edinburgh, 1965).
- ¹¹ Crawford, A. R., and Daily, B., *Nature*, **230**, 111 (1971).
- ¹² Crowell, J. C., *Bull. geol. Soc. Am.*, **68**, 993 (1957).
- ¹³ Bucher, W. H., in *Problems in Palaeoclimatology*, 3 (Interscience, London, 1963).
- ¹⁴ Piper, J. D. A., *Geophys. J. R. astr. Soc.* (in the press).
- ¹⁵ Piper, J. D. A., *Geophys. J. R. astr. Soc.*, **28**, 111 (1972).
- ¹⁶ Briden, J. C., *Seventeenth ann. Rep. Res. Inst. Afr. Geol. Univ. Leeds* (in the press).
- ¹⁷ Briden, J. C., *J. geophys. Res.*, **73**, 725 (1968).
- ¹⁸ Brock, A., *Nature*, **216**, 359 (1967).
- ¹⁹ Helsley, C. E., *Trans. Am. Geophys. Un.*, **46**, 67 (1965).
- ²⁰ Brock, A., *Geophys. J. R. astr. Soc.*, **24**, 303 (1971).
- ²¹ Piper, J. D. A., in *Implications of Continental Drift to the Earth Sciences*, 19 (Academic Press, London, 1973).
- ²² Irving, E., and Park, J. K., *Can. J. Earth Sci.*, **9**, 1318 (1972).
- ²³ Porath, H., *Earth planet. Sci. Lett.*, **2**, 409 (1967).
- ²⁴ Carey, S. W., in *Continental Drift—A Symposium*, 177 (Univ. Tasmania, Hobart, 1958).
- ²⁵ Embleton, B. J. J., *Earth planet. Sci. Lett.*, **17**, 217 (1972).
- ²⁶ Thompson, R., *Earth planet. Sci. Lett.*, **18**, 266 (1973).
- ²⁷ Germs, G. J. B., *Bull. 12* (Precambrian Research Unit, University of Cape Town, 1972).
- ²⁸ McElhinny, M. W., Briden, J. C., Jones, D. L., and Brock, A., *Rev. Geophys.*, **6**, 201 (1968).
- ²⁹ King, L. C., *Q. J. geol. Soc. Lond.*, **114** (1958).
- ³⁰ Harland, W. B., and Rudwick, M. J. S., *Scient. Am.*, **211**, 28 (1964).

Primordial Rare Gases in the Deep Earth

I HAVE suggested¹ that mantle-ambient rare gases are to be found in deep-sea basalts, trapped there by the same mechanism responsible for trapping excess ⁴⁰Ar. A previous search in such basalts dredged from an East Pacific seamount revealed gases similar to atmospheric in both elemental and isotopic ratios². I report here analyses of three Atlantic Ocean basalts whose elemental rare gas ratios are distinctly different from

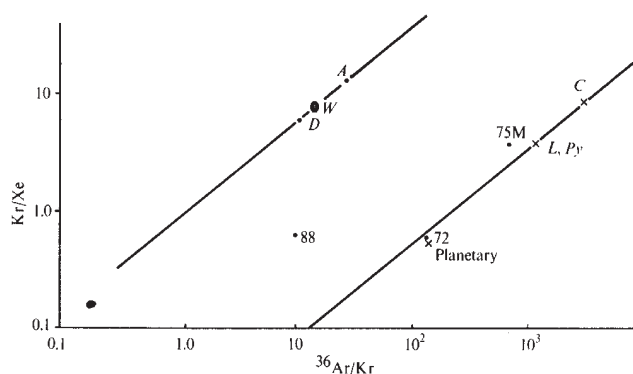


Fig. 1 Kr/Xe as a function of ³⁶Ar/Kr. The points A, W, D (defined in the text) define the values to be expected in a rock which samples the terrestrial atmosphere. The values C, L, Py, and Planetary define the values to be expected in a rock which samples primordial gases.

atmospheric, and which approximate primordial rare gases as seen in meteorites and the lunar surface.

The basalts studied here were dredged from along the Vema Fracture Zone, close to the Mid-Atlantic ridge. Samples 88 and 72 are glass rims, sample 75 M is an interior specimen. They will be described in detail elsewhere. Fission track ages and K/Ar ages on related rocks are consistent with their having erupted on the active ridge. Initial rare gas studies showed that these particular rocks contain large amounts of excess radiogenic ⁴He and ⁴⁰Ar, so they were expected also to have trapped the mantle-ambient non-radiogenic rare gases. Approximately 1 g chunks of whole rock were analysed. The data are presented in Table 1 and Figs 1 and 2.

The previous study² demonstrated that rare gases sorbed from an atmosphere-like reservoir will have Kr/Xe vs ³⁶Ar/Kr lying along a straight line on a log-log plot, as in Fig. 1. Here A is the atmospheric point, W represents several data on atmospheric rare gases dissolved in water, and D represents atmospheric gases adsorbed on the surface of wind-borne dust grains. Several data from the East Pacific seamount reported previously all lie along the line drawn through these three points. By contrast the primordial rare gases are shown by the point C, which represents cosmic abundances estimated by Cameron³; the point L, Py represents data obtained on lunar fine material⁴ and the meteorite Pesyanoe⁵ and constitutes the best available experimental approximation to solar wind gases. The point Planetary represents the so-called planetary component of the primordial rare gases⁶. The line through these points is parallel to the atmospheric line, implying a similar relationship between the planetary and solar components of the primordial rare gases.

Gases sorbed from an atmosphere-like reservoir of rare gases should lie along the upper line, as did those from the Pacific seamount and subaerial basalts; gases sorbed from a primordial-like reservoir should lie along the lower line, as do those from meteorites². Gases containing a mixture of the two components should lie in the area between the two lines. The data from the Atlantic Ocean rocks are labelled 88, 72 and 75 M.

The data clearly show large contributions from a non-atmospheric, primordial rare gas reservoir, most likely constituting the mantle region sampled by the magmas which have resulted in these rocks. The absolute amounts of these gases are shown in Table 1, as are meteoritic data. I note that in a rising magma the rare gases should be relatively enriched in the erupting material, so that the measured concentrations are an upper limit to the actual magmatic concentrations.

The ⁴He concentration is mostly radiogenic, resulting from the decay of U and Th before eruption. Therefore the measured ⁴He is only an upper limit to the primordial component. The Ne content was masked by high background levels, and so only upper limits were measured. These limits for ⁴He and Ne are distinctly below normalized solar or cosmic abundances

Table 1 Rare Gas Abundances in Atlantic Ocean Basalts

Sample	^4He	Ne	^{40}Ar	^{36}Ar	Kr	Xe
75 M	780	< 1.5	1.850	20.5	0.031	0.0080
72	19.8×10^4	< 1.9	7,000	1.67	0.013	0.023
88	24×10^4	< 30	33×10^4	3.75	0.38	0.62
Meteoritic planetary	$\sim 25 \times 10^4$	5–100	—	10^2 – 10^3	1–100	1–300
Meteoritic solar	$\sim 10^7$	10^4 – 10^5	—	10^3 – 10^4	5	~ 1

Ne, Kr, and Xe are given as total elemental abundances. Absolute abundances are accurate to $\sim \pm 20\%$, except for Ar $\sim \pm 5\%$. All data in units of 10^{-10} s.c.c. g^{-1} .

(Fig. 2) but cannot be distinguished from atmospheric or planetary values (except that the Ne in sample 75 M is distinctly below atmospheric). If the magmatic rare gas reservoir is solar or cosmic in character, it has strongly degassed its He and Ne while retaining the heavier rare gases.

Isotopic data are not as precise as one would wish due to the small sample sizes necessary for complete cleanup and to minor nonlinearities in the recording equipment. At present I can specify that all isotopic ratios are within $\pm 5\%$ of atmospheric. This accuracy is not sufficient to distinguish between atmospheric and primordial components, with the exception of ^{129}Xe which might be expected to show a large overabundance due to *in situ* decay of the extinct nuclide ^{129}I . No such anomaly was found.

The previous study² showed the rare gases trapped in an East Pacific seamount to be atmospheric; presumably the mantle source region had equilibrated its gases with the atmosphere. The work reported here on three active ridge basalts shows the rare gases to be more primordial in nature; presumably the mantle source region has never seen the terrestrial atmosphere. The East Pacific seamount was shown by fission track dating to have erupted far from the active East Pacific ridge⁷. If further data show this difference in character between the rare gases trapped in open basin seamounts and those trapped in active ridge flows to be a general one, it is likely to be indicative of basically different magmatic histories in the two cases. As such, it will obviously have great bearing on models of mantle upwelling and seafloor spreading.

These data contain also the germ of detailed inferences concerning the state of degassing of the inner Earth, its mode of formation, and rare gas fractionation processes involved in planetary formation. Further studies should delineate both

isotopic and elemental abundances and correlations (or lack of them) with ocean floor geography and plate tectonic models as well as with various mineralogical and eruptive parameters of the rocks involved.

This work was supported by the National Science Foundation; laboratory assistance was provided by Ted Middleton.

DAVID E. FISHER

Rosenstiel School of Marine and Atmospheric Science,
University of Miami,
Miami, Florida 33149

Received March 7, 1973.

¹ Fisher, D. E., *Earth planet. Sci. Lett.*, **12**, 321 (1971).

² Fisher, D. E., *Earth planet. Sci. Lett.*, **9**, 331 (1970).

³ Cameron, A. G. W., *Origin and Distribution of the Elements* (edit. by Ahrens, L. M.), 125 (Pergamon, 1968).

⁴ Marti, K., Lugmair, G. W., and Urey, H. C., *Science, N.Y.*, **167**, 548 (1970).

⁵ Marti, K., *Science, N.Y.*, **166**, 1263 (1969).

⁶ Pepin, R. O., and Signer, P., *Science, N.Y.*, **149**, 253 (1965).

⁷ Bonatti, E., and Fisher, D. E., *Earth planet. Sci. Lett.*, **11**, 307 (1971).

Contribution of Volcanic Sulphur Compounds to the Stratospheric Aerosol Layer

SULPHUR bearing compounds constitute a major portion of globally distributed persistent aerosol layer in the lower stratosphere^{1–3} and some considerations¹ indicate that the particles are formed *in situ*, presumably by the oxidation of gases containing sulphur. The relative importance of various terrestrial sources, however, has not been ascertained.

Here we report a study of sulphur concentrations and the $^{32}\text{S}/^{34}\text{S}$ isotopic ratios of selected stratospheric and tropospheric aerosol samples in which we compared the relative values and observed trends with the dates and locations of major volcanic eruptions. More details, including an assessment of the importance of other contributing sources, will be given elsewhere⁴.

Appropriate stratospheric aerosol samples, chosen with due regard to considerations of date, global location and height with respect to the tropopause, were obtained from existing libraries of the Health and Safety Laboratories of the USAEC and the Department of Defense. These samples were subjected to mass spectrometric analysis for isotopic enrichment; subsequently, concentration measurements were also made.

The results of our concentration measurements are shown in Fig. 1a. The times and approximate location of the important pyroclastic volcanic eruptions which have taken place during the past 10 yr in the equatorial latitudes are also indicated in Fig. 1a.

There is a clear correlation between sulphur concentration (expressed in μg of sulphate per standard m^3 of filtered air) and

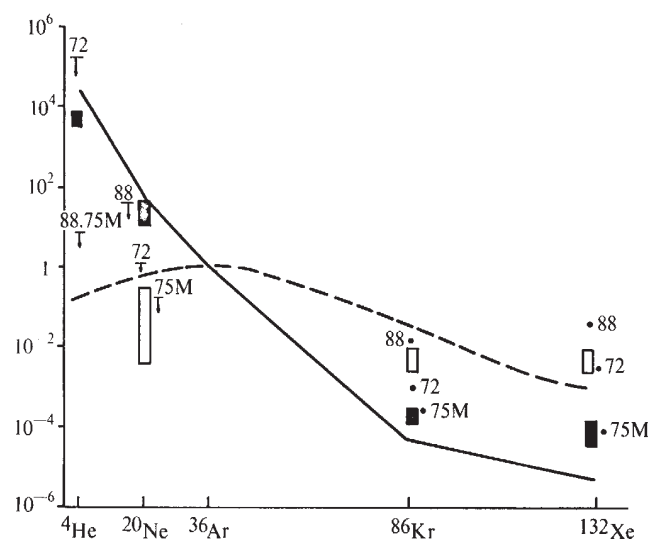


Fig. 2 Abundances of the rare gases normalized to $^{36}\text{Ar} = 1$. □, Planetary; ■, solar; —, cosmic; ---, atmospheric.

volcanic activity. Sulphate aerosol concentration in the Junge layer located south of the equator increased two orders of magnitude within a year after the catastrophic 1963 eruption of Mt Agung, at 8.5° S. Other data (not shown) reveal a similar effect for stratospheric aerosol samples collected during the same time period in the northern hemisphere from 1° N to 40° N. These data show that the sulphate contributions in the northern hemisphere from the eruptions of Agung, Taal, Fernandina, and Awu were all of about the same magnitude. Other samples collected in the Arctic (>55° N) stratosphere over the same time period and covering the eruptions of Trident (1963), Surtsey (1963), Sheveluch (1964), and Mt Redoubt (1966) exhibit the same dramatic increases in sulphate concentration.

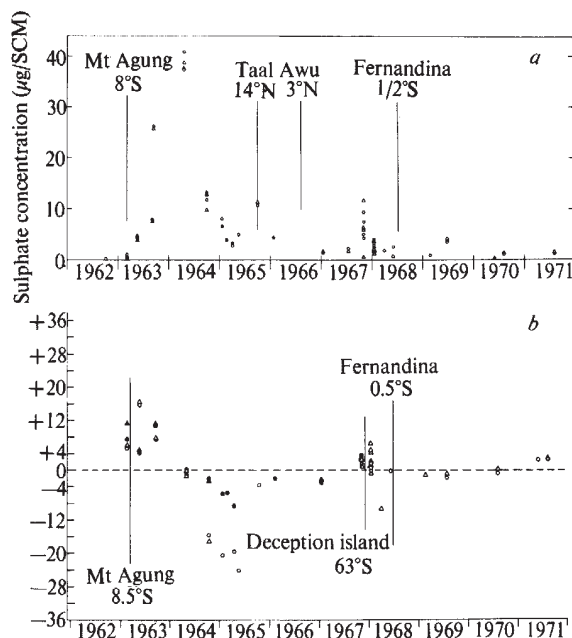


Fig. 1 *a*, Sulphate concentration as a function of time for stratospheric aerosols collected in the southern hemisphere (0° S to 43° S). *b*, Sulphur isotope ratios, $\delta^{34}\text{S}$, 0° S to 43° S. All samples are corrected for trace sulphur background of the filter media. Δ , Corrected data where primary blank was available for direct determination; $\circ$, cases where an average blank was used.

Isotopic ratios for the southern hemisphere aerosols are plotted in Fig. 1*b*. The cyclic trends in $\delta^{34}\text{S}$ values of samples collected subsequent to the eruptions of Agung and Fernandina are typical of those observed for samples taken in the north equatorial and Arctic stratosphere after the other eruptions mentioned. These data provide additional evidence for the source of the sulphate. To interpret the results, due consideration must be given to the isotopic composition of the tephra, as well as changes likely to result from gas-to-particle chemical reactions occurring during the residence of the gases after injection into the stratosphere.

The positive $\delta^{34}\text{S}$ values of the stratospheric samples collected shortly after the eruptions undoubtedly result from a weighted average of the value for the injected tephra, together with the limited quantity of particles formed by the conversion of gases to particles in the rising plume, and pre-existing material in the stratosphere. Several volcanic tephra samples were obtained from the Smithsonian Institute for recent eruptions of Hekla, Mt Mahon, Mt Arenal and Kilauea. Although not necessarily representative of all tephra, in each of these cases the $\delta^{34}\text{S}$ values were positive.

The subsequent abrupt decline in $\delta^{34}\text{S}$ value with time indicates a depletion of the ^{34}S isotope with respect to meteoritic sulphur for the aerosol particles being sampled. Such a change in ^{34}S enrichment of stratospheric sulphates suggests a frac-

tionation of the isotopes of sulphur during exposure to a reactive chemical environment, and is thus indicative of chemical change. The direction that this change should take is difficult to assess theoretically, but a decrease in $\delta^{34}\text{S}$ value for the unreacted gas has been observed² experimentally and is also indicated by our results.

Within a month after an eruption, most of the coarse tephra has settled below the stratospheric sampling regions selected for this study. Therefore, we expect subsequent sampling to collect increasing amounts of particles produced from gases which were injected by the rising plume, as well as limited quantities of pre-existing particles settling from upper altitudes. Although the average residence time for particles in the stratosphere is about 1.5 yr, the average time of a particle spent at a particular altitude sample, even with due allowances for sampling height variations between missions, is comparatively short. Consequently, particles collected at progressively later times following an eruption represent those formed at higher altitude which have eventually settled into the sampling region.

As the injected gases diffuse upward, they are continually converted to particles from gas whose $\delta^{34}\text{S}$ value is progressively decreasing. Eventually, the gas converts to particulate matter; particles with progressively lower $\delta^{34}\text{S}$ values become concentrated at the higher altitudes. Because the upper layer particles require a longer settling time, the $\delta^{34}\text{S}$ value for samples collected in the lower stratosphere would be expected to show an increase followed by a decrease for some period of time after an eruption. After several years without a large eruption, the stratospheric isotopic ratio would be expected to return to an average value indicative of other contributing sources; such an effect is always observed, the average $\delta^{34}\text{S}$ value being 2.6 ± 0.3 (our unpublished work with A. L. Lazrus).

This is confirmed by the data of Fig. 1. The solid points represent values of samples collected in the stratosphere at a lower (15.3 km) altitude between 40° S and 43° S, and the open points are samples collected at higher altitudes ranging from 18.3 to 19.8 km. Although the sulphate concentration values of these samples are in good agreement, the greater degree of ^{34}S enrichment for the lower altitude data is apparent.

So volcanic emissions represent the principal source perturbing the sulphate concentration of the stratosphere. Additional results⁴ provide some evidence that upwelling Hadley cell circulation at the equator is an important source for that portion of the layer which persists in the absence of volcanic eruptions.

This research was performed under the auspices of the US Atomic Energy Commission and was supported by the Environmental Protection Agency under an interagency agreement. We thank Mr P. Krey (HASL-AEC) and individuals in the DOD for help in obtaining samples; Professors C. Junge (MPI-Mainz), R. Newell (MIT), and J. Friend (NYU) for discussions; and Dr L. Newman and Mr J. Forrest for performing the concentration and $\delta^{34}\text{S}$ value analyses.

A. W. CASTLEMAN, JUN.
H. R. MUNKELWITZ
B. MANOWITZ

Department of Applied Science,
Brookhaven National Laboratory,
Upton, LI, New York 11973

Received April 20, 1973.

- 1 Junge, C. E., Chagnon, C. W., and Manson, J. R., *J. Meteorol.*, **18**, 81 (1961).
- 2 Friend, James P., *Tellus*, **18**(2), 465 (1966).
- 3 Lazrus, A. L., Gandrud, B., and Cadle, R. D., *J. geophys. Res.*, **76**, 8083 (1971).
- 4 Castleman, jun., A. W., Munkelwitz, H. R., and Manowitz, B., *Tellus* (in the press).
- 5 Tucker, W. D. (ed.), *The Atmospheric Diagnostic Program at Brookhaven National Laboratory: Third Status Report*, BNL-50280 (1970).

Accurate Position of GX5-1 from Lunar Occultations

HERE we report the first observations of a lunar occultation of an X-ray source using detectors on board an Earth satellite. The source GX5-1 has now been located with an error box (95% confidence) of 6×20 arc s. A 2σ upper limit of 8 arc s has been put on the source diameter.

The source GX51 (2U1757-25) is the brightest of a class of powerful X-ray emitting objects lying in the galactic plane and concentrated in the direction of the galactic centre. Study of these objects, which are responsible for a large fraction of the total X-ray emission from our Galaxy, is impeded by the lack of any optical identification. The smallest error limits for the position of GX5-1 published so far¹ (~ 1.5 arc min) include a large number of stars and a weak radio source², which has been suggested as a possible counterpart.

The object GX5-1 is at present undergoing a series of lunar occultations³. Calculations of the track of the satellite Copernicus, which carries several X-ray telescopes constructed at MSSL, showed that on four occasions during the period March to August 1973, the satellite passes through the X-ray shadow of the Moon with respect to GX5-1 (using the position of GX5-1 as given by Schnopper *et al.*¹). Both the disappearance (D1) and the reappearance (R1) of GX5-1 were observed on March 26, 1973; on April 22, 1973, although both disappearance (D2) and reappearance (R2) were visible to the spacecraft, the reappearance unfortunately occurred during the dead time of the spacecraft data-handling system. The observations thus yield three position lines on the celestial sphere whose common intersection is the most probable position of GX5-1.

The counts from each telescope (4 to 12 keV detector, 14 cm^2 , 2×5 arc deg field of view, mechanical collimator; 1.4 to 4.2 keV detector, 3.7 cm^2 , 12 arc min field of view, grazing incidence paraboloidal mirror; energy ranges are nominal, from preflight calibration) are accumulated in 62.9 s frames before being read out, successive frames being separated by a dead-time of 23.6 s. For the April 22 observations, the accumulated counts were read out every quarter frame, with no dead time between those subframe readouts.

Because GX5-1 is a strong source, the count rate from this source was far larger than the background count rate, both from the diffuse X radiation and charged particles, as measured while GX5-1 was occulted. A count intermediate between the background and full source rate was accumulated in those frames in which each D or R occurred. The high source to background ratio allowed an accurate determination of the fraction of a frame during which GX5-1 was in view. In this way timing accuracy much better than the frame duration was possible.

Background counts from charged particles were subtracted from all data before any further analysis (A. C. Fabian, private communication). The statistical uncertainty in the number of counts in each D or R frame dominated all other sources of error.

An upper limit to the size of a source can be derived from quarter-frame data because the occultation is observed in a single quarter-frame rather than spread out over several frames. The derived occultation time, for an extended source, would correspond to the position of the centroid of X-ray

emission. The half width of the source would correspond to the time between the occultation and the beginning of the frame (or end, whichever is closer). This must be increased by the quadratic sum of the 2σ error in the occultation time and the maximum time into the adjacent frame that the start or end of an occultation of a diffuse source could have occurred and been masked by a 2σ statistical deviation.

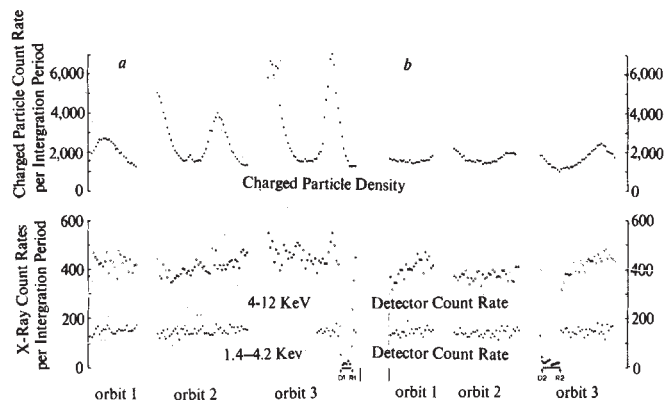


Fig. 1 Count rates of the 4 to 12 keV and 1.4 to 4.2 keV X-ray detectors before, during and after the lunar occultations on March 26, 1973 (a), and April 22, 1973 (b). Charged particle background has been subtracted. Also shown is the charged particle density as measured by the anticoincidence guard rate. Each point records the counts in a single integration period (62.9 sec). The points lie at 86.5 s intervals. The breaks in the data of each day represent GX5-1 going behind the Earth. The orbital period is 100 min.

The average source rate is constant in the 1.4 to 4.2 keV detector before and after each occultation (Fig. 1). All data obtained from this detector were used. The data from the 4 to 12 keV detector show marked secular variations. The derived event time turns out to be relatively insensitive to the choice of data used in determining the full source rate, but to reduce chances of systematic error only data observed directly before the occultations were used.

The Goddard Space Flight Center provided accurate orbital information which was combined with the time analysis described above to give the position of the satellite when each disappearance or reappearance was observed (listed, with error limits, in Table 1). These data, together with the lunar ephemeris in present use at the Royal Greenwich Observatory, were used to calculate the three positions of the lunar limb as viewed from the satellite at the appropriate times, D1, R1 and D2. A small correction of $+0.4$ arc s, taken from Watts's charts⁴ of the marginal zone of the Moon, was applied to the position line to allow for the unevenness of the profile. In Fig. 2 the $\pm 2\sigma$ error bounds of these positions are superimposed on an enlargement of the red Palomar Sky Survey print of the area. The contribution to these error bounds from the uncertainty in the satellite's longitude was minimized because on all three events the Moon was near the satellite's horizon, and

Table 1 Observation Times and Satellite Geodetic Coordinates with Standard Deviations (1σ)

Event	Date	UT (detector 1)	UT (detector 2)	UT (average)	Longitude (W)	Latitude (N)	Height (km)
D1	March 26, 1973	12 h 56 min 17.3 s ± 3.2 s	12 h 56 min 18.0 s ± 4.0 s	12 h 56 min 17.7 s ± 2.4 s	48.54° $\pm 0.03^\circ$	34.44° $\pm 0.01^\circ$	745.5 ± 0.5
R1	March 26, 1973	13 h 04 min 58.6 s ± 4.0 s	13 h 05 min 02.2 s ± 5.4 s	13 h 05 min 00.4 s ± 3.1 s	15.83° $\pm 0.03^\circ$	25.20° $\pm 0.01^\circ$	741.4 ± 0.5
D2	April 22, 1973	16 h 35 min 28.5 s ± 0.7 s	16 h 35 min 30.4 s ± 1.2 s	16 h 35 min 29.4 s ± 0.8 s	285.70° $\pm 0.03^\circ$	34.85° $\pm 0.01^\circ$	737.5 ± 0.5

therefore the satellite's motion in longitude was mainly directed towards the Moon. The 2σ error in latitude of $\pm 0.02^\circ$ contributed about ± 1 arc s to the error bounds in Fig. 2. The area bounded by the intersection of the error bars is the best position obtainable from these occultation observations.

This position lies within the error circle given by Schnopper *et al.*¹ and on the edge of the 3U error box (R. Giacconi, private communication). None of the numbered stars in Fig. 1 of Kunkel *et al.*⁵ lies within the new error box.

Table 2 Four Corners of Common Intersection Area in Fig. 3

RA (1950.0)	Dec (1950.0)
17 h 58 min 03.	$-25^\circ 04' 39''$
03.4 s	41"
04.4 s	29"
04.9 s	16"

The occultation data set a 2σ upper limit of 8 arc s on the angular diameter of GX5-1, substantially below the limit given by Schnopper *et al.*¹. The 3U catalogue (R. Giacconi, private communication) reports variability by a factor of 2, indicating that GX5-1 is likely to be a compact source. Our observations showed variations of up to 38% from day to day and up to 10% in a given day (Fig. 1). Rappaport *et al.*⁶ have analysed data on GX5-1 for fast time variations and have set an upper limit of 3% on the pulsed fraction over the period range 10 ms to 15 s.

Figure 3 is a diagram of the region of intersection of the 2σ occultation bounds together with the positions of nearby stars and a faint radio source. The positions of stars 21 and 22 and

the radio source are taken from Braes *et al.*². The positions of stars 7, 23 and 24 were determined at the RGO on a photographic negative of the Palomar chart printed onto a glass plate and measured in the usual way, using several bright field stars to derive the scale and orientation of the plate. The positions so derived have a 2σ error on ± 2 arc s. There are no objects near the 2σ occultation limits on the Palomar red plate other than those shown in Fig. 3. Star 23 is not visible on the blue plate, 24 is just visible, and 7, 21 and 22 are clearly visible.

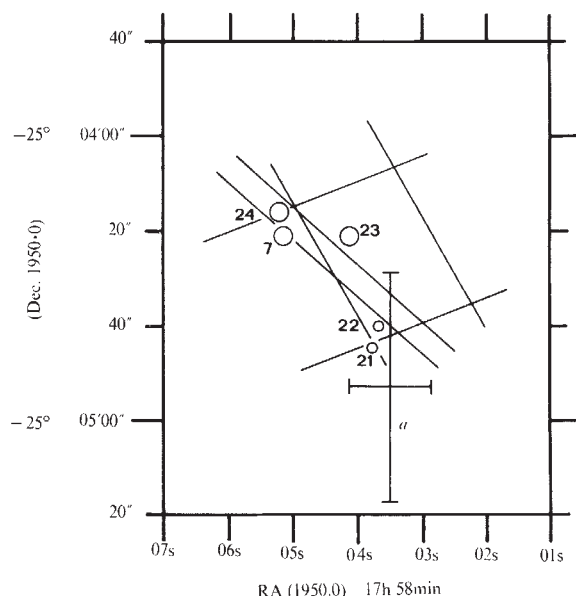


Fig. 3 Diagram of the intersection region in Fig. 2. $\circ$, Measured star positions down to the limiting magnitude of the Palomar print. The positions for 21 and 22 are due to Braes *et al.*² and those for 7, 23 and 24 were measured at the RGO as described in the text. The position of the faint radio source given by Braes *et al.*² is also shown (a). All error bounds in the figure are at the $\pm 2\sigma$ level.

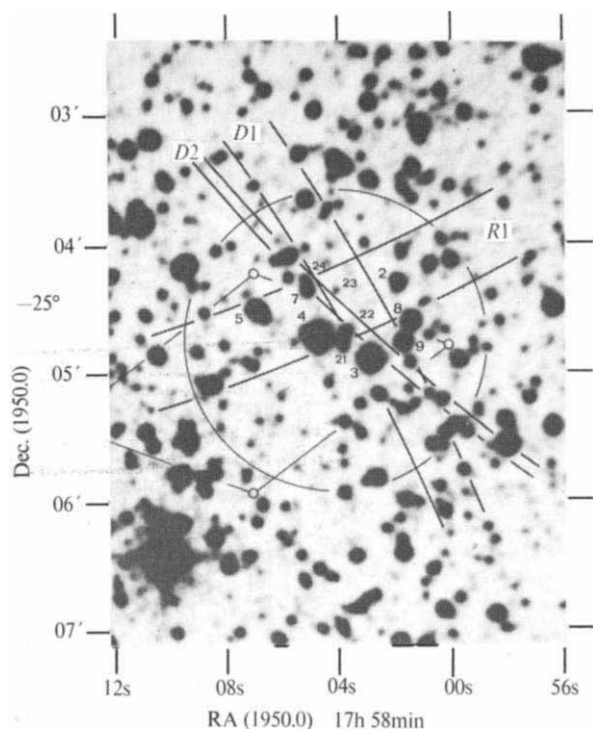


Fig. 2 Enlargement of Palomar Survey red print (copyright National Geographic Society) showing the intersection of the $\pm 2\sigma$ limits for the three occultation events D1, R1 and D2. The error circle of radius 1.2 arc min due to Schnopper *et al.*¹ and the 3U 90% limits (private communication), represented by small open circles, are also shown. Star numbers up to and including 9 are those assigned by Kunkel *et al.*⁵; we have assigned numbers 21 to 24. Star 23 is close to the limiting photographic magnitude of 20 mag on the original print.

Although the lack of a conspicuous object in the error box is disappointing, it is not unexpected if GX5-1 really is near the galactic centre. A distance of 12 kpc is implied if the low energy cutoff of GX5-1 is caused by absorption through an interstellar column density $N_H = (2.5 \pm 0.5) \times 10^{22} \text{ H cm}^{-2}$ (refs. 6 and 7). Tarengi and Reina (ref. 8 and private communication) show that the interstellar dust associated with this amount of gas would produce a visual extinction $A_v = 15 \pm 3$ mag. But if GX5-1 were associated with a binary system whose primary was a BO star with $M_v = -6$ there would be a possibility of seeing it. The secondary in the system would be too faint to be detected, but the primary, while not present on a Palomar Survey blue plate, would be at the limiting magnitude of the corresponding red plate. This model would make the proximity of stars 7, 21, 22 and 24 to the error box probably fortuitous.

We note the similarity between GX5-1 and GX3+1. Both are bright, variable X-ray sources with low energy cutoffs indicating distances on the order of 10 kpc. The revised lunar occultation position⁹ of GX3+1 shows no bright stars in the error box but lies near a weak radio source, just like GX5-1.

The maximum visibility of the double star system described above would be at a wavelength $\geq 2 \mu\text{m}$. Thus the best future prospects for optical identification of GX5-1, GX3+1, and presumably the other similar galactic centre X-ray sources, would be an extension of the optical search to infrared wavelengths and simultaneous X-ray, infrared and radio observations to look for correlated time variations.

J. A. H. is a NATO postdoctoral fellow. P. J. N. D. is an SRC postdoctoral fellow.

J. A. HOFFMAN

*X-ray Astronomy Group,
Department of Physics,
University of Leicester*

P. J. N. DAVISON

*Mullard Space Science Laboratory,
University College, London*

L. V. MORRISON

*Nautical Almanac Office,
Royal Greenwich Observatory*

Received June 7, 1973.

¹ Schnopper, H. W., Bradt, H. V., Rappaport, S., Bougham, E., Burnett, B., Doxsey, R., Mayer, W., and Watt, S., *Astrophys. J. Lett.*, **161**, L161 (1970).

² Braes, L. L. E., Miley, G. K., and Schoenmaker, A. A., *Nature*, **236**, 392 (1972).

³ Morrison, L. V., *Nature phys. Sci.*, **229**, 192 (1971).

⁴ Watts, C. B., *Astr. Pap. XVII* (Washington, 1963).

⁵ Kunkel, W., Osmer, P., Smith, M., Hoag, A., Schroeder, D., and Hiltner, W. A., *Astrophys. J. Lett.*, **161**, L169 (1970).

⁶ Rappaport, S., Bradt, H. V., Naranan, S., and Spada, G., *Nature*, **221**, 428 (1969).

⁷ Seward, F. D., Burginyon, G. A., Grader, R. J., Hill, R. W., and Palmieri, T. M., *Astrophys. J.*, **178**, 131 (1972).

⁸ Tarengi, M., and Reina, C., *Nature phys. Sci.*, **240**, 53 (1972).

⁹ Janes, A. F., Pounds, K. A., Ricketts, M. J., Willmore, A. P., and Morrison, L. V., *Nature*, **244**, 349 (1973).

looked to be a serious optical candidate has been shown² to possess no unusual properties which could indicate that it had a strong X-ray emission.

Recently the original X-ray data have been checked and the discovery of a timing error has resulted in a shift of about 2.5 s (RA) in the MSSL position. This is equivalent to moving the intersection position about 1 arc min along the Leicester error box; the new position is shown in Fig. 1.

There is no optical object in the new overlap position; Kunkel's objects "2" and "3" (ref. 3) lie too far away to be considered. A search for a radio counterpart at the Leiden Observatory (G. K. Miley, private communication) has shown that a weak source (11 ± 4 m.f.u. at 1,415 MHz) exists at RA 17 h 44 min 50.15 $\pm$ 0.2 s, Dec—26° 33.0' $\pm$ 0.25' (1950). This source, with its 2 σ error bars, is shown in Fig. 1. It is very close to the centre of the error box quoted by Schnopper *et al.*⁴ and is of similar strength to possible radio candidates for GX5-1 (10 ± 3 m.f.u.)⁵ and Cyg X-1 (24 ± 4 m.f.u.)⁶.

A. F. JANES

K. A. POUNDS

M. J. RICKETTS

*X-Ray Astronomy Group,
Department of Physics,
University of Leicester*

A. P. WILLMORE *

*Mullard Space Science Laboratory,
University College, London*

L. V. MORRISON

*Nautical Almanac Office,
Royal Greenwich Observatory,
Herstmonceux Castle,
Hailsham, Sussex*

Received June 8, 1973.

* Present address: Department of Space Research, University of Birmingham.

¹ Janes, A. F., Pounds, K. A., Ricketts, M. J., Willmore, A. P., and Morrison, L. V., *Nature phys. Sci.*, **235**, 152 (1972).

² Rodgers, A. W., *Nature*, **237**, 273 (1972).

³ Kunkel, E., Osmer, P., Smith, M., Hoag, A., Schroeder, D., Hiltner, W. A., Bradt, H., Rappaport, S., and Schnopper, H. W., *Astrophys. J. Lett.*, **161**, L169 (1970).

⁴ Schnopper, H. W., Bradt, H. V., Rappaport, S., Bougham, E., Barnell, B., Doxsey, R., Mayer, W., and Watts, S., *Astrophys. J. Lett.*, **161**, L161 (1970).

⁵ Braes, L. L. E., Miley, G. K., and Schoenmaker, A. A., *Nature*, **236**, 392 (1972).

⁶ Braes, L. L. E., and Miley, G. K., *Nature phys. Sci.*, **232**, 246 (1971).

BIOLOGICAL SCIENCES

Effects of Contraceptive Pill Constituents on Foetal Mouse Hearts

ALTHOUGH the use of the contraceptive pill carries a slight risk, it is generally felt that the harmful side effects are outweighed by the benefit of avoiding unwanted pregnancies. Following the thalidomide tragedy, there is a wider appreciation of the risk of substances taken during pregnancy injuring the foetus. As we now have a direct method of studying the action of drugs

Correction to the Position of GX3+1

WE have previously given the results of two lunar occultations¹ of the X-ray source GX3+1. Only two optical objects were nearby, both outside the error box. The only one of these which

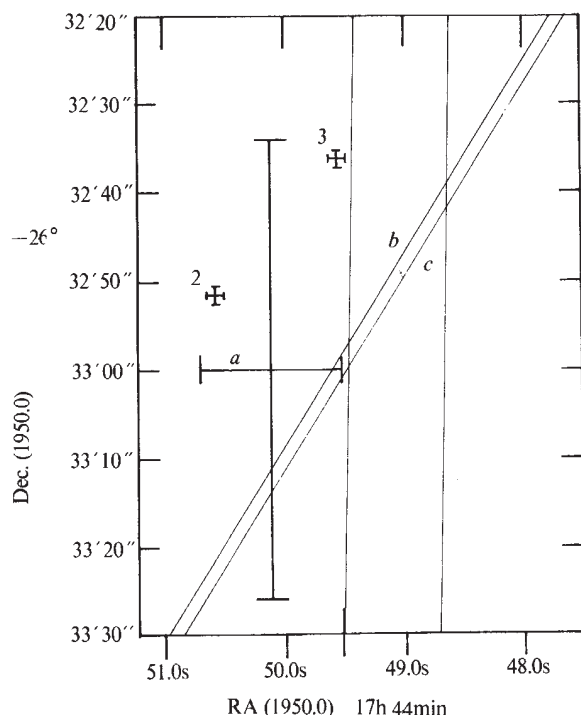


Fig. 1 The lines represent the 2 σ error limits of the positions of the source obtained from each experiment; the radio and optical sources also have error limits of 2 σ shown to their positions. *a*, Radio source; 2 and 3, Kunkel's objects³; *b*, Leicester observations; *c*, MSSL observations.

on the foetal heart¹, we felt that it was advisable to see whether a pregnant woman who started taking the pill would jeopardize her foetus.

In this initial study we took foetal hearts from pregnant mice and cultured them by the well established technique instituted by H. Fell and consolidated by K. Wildenthal². Eight groups of twenty mouse hearts were used to test hormones commonly used in the pill at the probable achieved concentrations. Forty control hearts were used and a further twenty hearts were used to check that the ethanol solvent for the hormones did not affect the results.

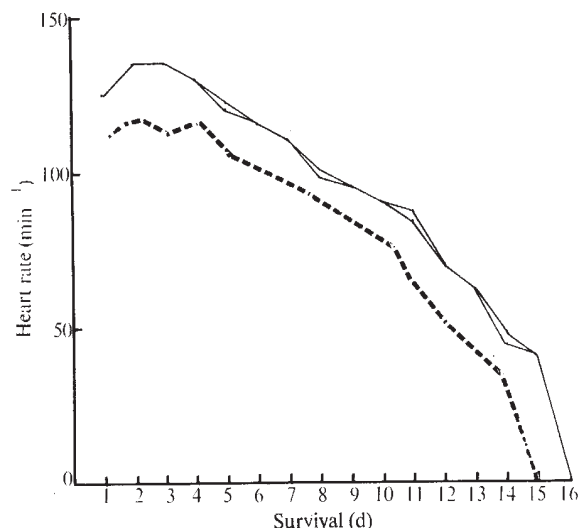


Fig. 1 The enhancing effect and increased survival of hearts cultured with 1 µg of ethinyloestradiol.

Following a previous technique³, 9 and 15 d foetuses were removed aseptically from freshly killed pregnant mice. The heart and lungs were removed and transferred to sterile Petri dishes under a dissecting microscope. The heart was dissected from the lungs, the pericardium and blood vessels were carefully trimmed away and residual blood was washed from the organ with culture medium. The isolated hearts were then placed on a stainless steel grid, the larger ones directly and the smaller ones on sterile filter paper (Whatman No. 1), so that the lower side of the organ was exposed to 2 ml of culture medium in the 30 mm × 13 mm Petri dishes and the top to the atmosphere. The small Petri dishes were placed in 90 mm × 13 mm Petri dishes, a filter paper soaked with water placed on the bottom to increase humidity and they were covered with a loose fitting lid. The cultures were placed on racks in airtight McIntosh jars filled with an atmosphere of 95% oxygen and 5% carbon dioxide⁴. They were kept in incubators at 37° C ± 0.5° C. The medium and atmosphere were renewed every 24 h.

The hormones tested were ethinyloestradiol (1 µg and 10 µg), norgestrel (1 µg and 10 µg), norethisterone acetate (1 µg and 10 µg), a combination of 1 µg ethinyloestradiol and 1 µg norethisterone acetate and a combination of 10 µg ethinyloestradiol and 10 µg norethisterone acetate.

The eight hormones, in 0.2 ml of 1% ethanol, were added to a medium which consisted of 199 medium (65%), foetal calf serum (35%, Wellcome Reagents Ltd), cortisol (0.1 µg ml⁻¹), insulin (50 µg ml⁻¹), penicillin (100 units ml⁻¹) and streptomycin (0.1 mg ml⁻¹) to total 100 ml.

Ethanol (0.02 ml%) caused no demonstrable change in the beating or survival period of the hearts, when compared with the controls. Ethinyloestradiol (1 µg) in 100 ml produced an increase in heart rate and a 1 d increase in survival time when

added to the culture medium. Ethinyloestradiol (10 µg) had less effect on the rate than 1 µg with no increase in survival. Norgestrel (1 µg) added to the culture medium caused an increase in rate, an obvious increase in the force of contraction of both atria and ventricles, and a 3 d prolongation of the survival period.

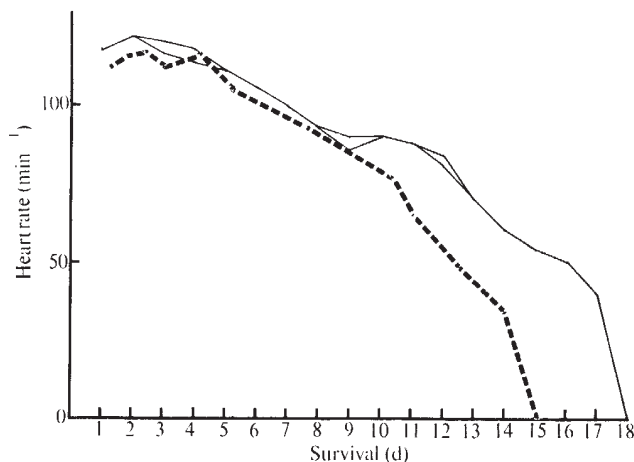


Fig. 2 Increased survival time with little early increase in beating, with 1 µg norgestrel.

Norgestrel (10 µg) also affected the hearts, increasing their rates, force of contraction and survival period, but to a lesser extent than 1 µg and prolonging survival by only 1 d. Norethisterone acetate (1 µg) caused an overall slowing of the heart rates and force of contraction and a 1 d reduction in survival time. Norethisterone acetate (10 µg) produced a definite decrease in rate and force of contraction of atria and ventricles at all stages, as well as a 3 d shortening of survival period. At this concentration, norethisterone acetate had a greater depressant effect than the 1 µg concentration. Ethinyloestradiol (1 µg) and 1 µg norethisterone acetate appeared to antagonize each other, resulting in a performance similar to the controls. Ethinyloestradiol (10 µg) and 10 µg norethisterone acetate decreased the rate compared with controls.

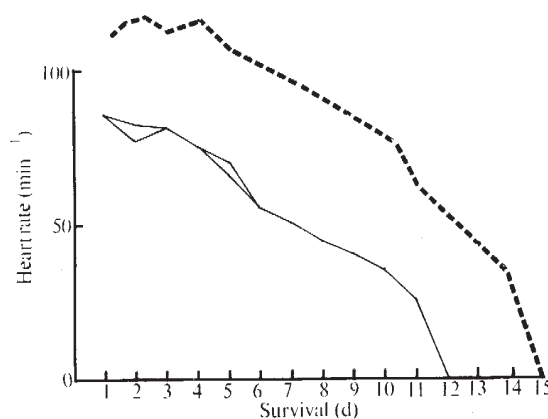


Fig. 3 Profound depressant effect and reduction in survival time with 10 µg norethisterone acetate.

Of the individual hormones studied, 1 µg norgestrel had the greatest enhancing effect on survival and performance whilst 10 µg norethisterone acetate had the greatest depressant effect

on both parameters. The lesser enhancing effects of ethinyloestradiol appear to virtually cancel the depressant effects of norethisterone acetate in the concentrations studied. This combination was chosen for study because, in various proportions, it is the commonest used in the formulation of contraceptive pills. Norgestrel is less commonly used as "Progestogen", in combination with oestrogens, in the pill and so was not studied in combination with them.

Table 1 Performance of Foetal Hearts Exposed to Test Hormones Compared with Controls

Substance	Survival	Rate	Force of contraction
Solvent	No detectable change		
1 µg Ethinyloestradiol	+1 d	↑	—
10 µg Ethinyloestradiol	—	↑	—
1 µg Norgestrel	+3 d	↑↑	↑↑
10 µg Norgestrel	+2 d	↑↑	↑↑
1 µg Norethisterone acetate	—	↓	↓
10 µg Norethisterone acetate	-2 d	↓↓	↓↓
1 µg Ethinyloestradiol + 1 µg norethisterone acetate	—	—	—
10 µg Ethinyloestradiol + 10 µg norethisterone acetate	—	↓	—

Number and direction of arrows indicate magnitude and type of response.

If inferences can be drawn from this preliminary study using mouse foetal hearts, it would seem that the concentration of norethisterone acetate in the pill should be kept to a minimum. When the current problems of obtaining human foetal material are overcome, this study will be repeated using human foetal hearts.

We thank the Board of Governors of the National Heart Hospital and the British Heart Foundation for support. We also thank Dr E. Lockey, Miss D. M. Hughes, and S. R. Armstrong. M. M. H. was, during this study, a WHO fellow at the National Heart Hospital.

MAHMOUD MOSTAFA HASSAN

Institute of Cardio-Thoracic Surgery, Cairo

DONALD B. LONGMORE

*National Heart Hospital,
Westmoreland Street, London W1*

Received April 11; revised June 8, 1973.

¹ Armstrong, S. R., and Longmore, D. B., *Nature*, **243**, 350 (1973).

² Wildenthal, K. J., *Am. J. Physiol.*, **221**, 238 (1971).

³ Hughes, D. M., and Longmore, D. B., *Nature*, **235**, 334 (1972).

⁴ Nagler, J., and Longmore, D. B., *Nature*, **242**, 197 (1973).

Enhancement of Metabolic Coronary Dilatation by Aspirin-like Substances by Suppression of Prostaglandin Feedback Control?

In the isolated, perfused heart, increased activity induced by catecholamines and/or calcium ions is followed by enhanced coronary flow, attributable to some metabolic process in the myocardium. This metabolically induced coronary basodilatation (MCD) is inhibited by prostaglandin E_1 (ref. 1). The

increased MCD response correlates with 3'5'-cyclic AMP levels. Furthermore, when prostaglandins (PGs) are infused, inhibition of MCD correlates with diminished cyclic AMP production^{2,3}. The inhibition of MCD by exogenous administration of PGs suggests that these autacoids may play a role as modulators of the coronary reaction to increased cardiac activity⁴. If it were possible to demonstrate the participation of PGs produced endogenously, their relevance in this modulatory mechanism of adaptation of coronary flow would become evident.

As indomethacin and aspirin have been shown to inhibit PG synthesis⁵⁻⁷, we investigated the actions of these substances on the MCD which follows the increase of cardiac activity produced by noradrenaline, Ca^{2+} (ref. 8) and tachycardia.

Male Wistar rats, weighing 240–265 g, were anaesthetized with ether and the hearts were isolated and perfused by the Langendorff method at 50 mm Hg perfusion pressure. The Krebs-Henseleit bicarbonate perfusate contained one half the usual calcium concentration⁹ and was gassed with $O_2 : CO_2$ (95 : 5). Phentolamine ($400 \mu g l^{-1}$) was added to the perfusate to inhibit an adrenoceptive action of noradrenaline. The force of contraction was measured by attaching a clip to the ventricular apex and connecting it to a force displacement transducer (Grass FT.03) under 2 g initial tension and coronary flow was measured continuously by a modified flowmeter¹⁰ and recorded on a polygraph. The interventricular septum was clamped and, with a dipolar electrode placed on the surface of the right ventricle, the heart was stimulated at 210 beats min^{-1} with square wave pulses (5 ms duration, current approximately 20% above threshold). By pacing the heart it was possible to achieve uniform cardiac activity and also to avoid changes of heart rate due to noradrenaline. The effect of tachycardia was studied by pacing the heart at 250 beats min^{-1} for 30 s.

Increments in coronary flow produced by noradrenaline, Ca^{2+} , adenosine or tachycardia were assessed by planimetry of the area under the flowmeter tracing from the beginning of dilatation until the point at which the coronary flow had returned half way from maximum response to control level (Fig. 1). At the end of each experiment, of approximately 60 min duration, the heart was removed, blotted and weighed (mean wet weight 1.01 ± 0.024 g). Single doses of noradrenaline (7 ng and 9 ng), $CaCl_2$ (10 µg and 20 µg) and adenosine (0.2 g) in volumes of 0.1 ml were administered by aortic cannula. Aspirin and indomethacin were infused at the rate of 0.1 to 0.2 ml min^{-1} while the PGE_2 was added to the perfusate reservoir. Significance of changes was calculated by the *t*-test for paired data.

The basic coronary flow for the series, shown in Fig. 2, was 6.8 ± 0.25 mg $g^{-1} min^{-1}$. Administration of noradrenaline caused dose dependent inotropic effects and proportional increases in MCD. In most of the experiments, coronary dilatation developed without a preceding decrease in flow (Fig. 1a). In spite of the presence of phentolamine, a phase of reduced flow preceded the dilatation in some experiments (Fig. 1b). This reduced flow is attributed to the increased "extravascular support" leading to the increased impedance to flow¹⁰. Similar observations were made on the administration of Ca^{2+} or during tachycardia (Fig. 1c, d). The initial reduction of coronary flow in response to Ca^{2+} could also be attributed to both vascular smooth muscle contraction¹¹⁻¹³ and to increased "extravascular support"¹⁴.

Aspirin was infused at a rate of 90–108 µg min^{-1} and indomethacin at 10–40 µg min^{-1} . Assuming the mean control coronary flow rate, the final drug concentrations in the perfusate were 0.076 to 0.15 mM and 0.0042 to 0.017 mM, respectively. These concentrations are of the same order as those found to be effective in inhibiting synthesis of $PG^{5,15-17}$. The basic coronary flow during infusions of aspirin was 6.6 ± 0.25 ml min^{-1} , and with indomethacin at 10–20 µg min^{-1} , it was 6.6 ± 0.52 ml min^{-1} . At the highest dosage of indomethacin, 40 µg min^{-1} , there was at first a gradual increase in

coronary flow that later subsided to 6.5 ± 0.17 ml min⁻¹. After only 5–10 min infusion of either drug, a clear potentiation of MCD responses to challenging doses of noradrenaline, Ca²⁺ or to tachycardia was observed at all concentrations studied. The effects of full potentiation obtained after approximately 20 min of infusion are shown in Fig. 1 and the data for the series are tabulated in Fig. 2.

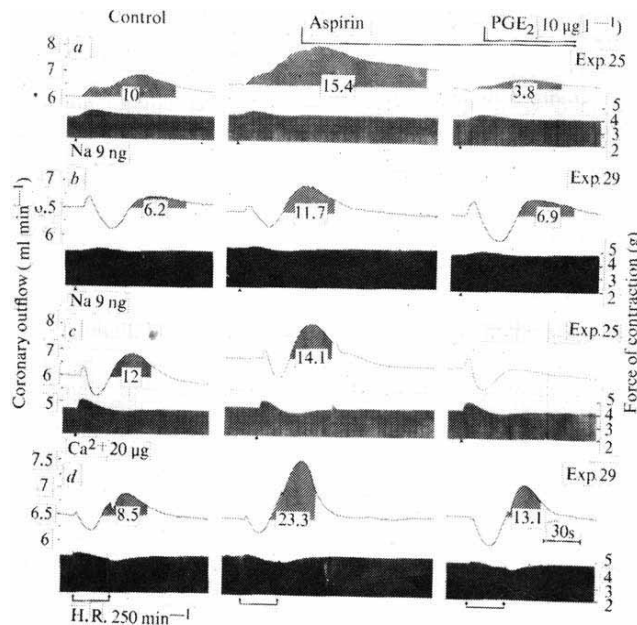


Fig. 1 Effects of aspirin before and during PGE₂ infusion on MCD responses. The calibrations on the left refer to coronary outflow, the hatched portion represents the area measured for MCD responses and the numbers within the hatching denote % change from control level. The right hand calibration represents the force of heart contractions. Doses of noradrenaline (Na) and calcium chloride (Ca²⁺) were given (▲). Tachycardia (250 beats min⁻¹) was applied during the interval (↑).

Aspirin and indomethacin affected only the MCD response, as cardiac hyperactivity produced by noradrenaline, Ca²⁺ or tachycardia was not affected. In a few experiments where the aspirin infusion was stopped, the enhanced MCD response continued for over 45 min.

The enhancement of MCD by aspirin or indomethacin was so predominant that a less pronounced phase of initial decrease of flow was observed (Fig. 1b, c, d). In the absence of this initial decreased flow the normal MCD response is enhanced to a greater extent (Fig. 1a).

When stable potentiation of MCD was reached, perfusion with PGE₂ at a concentration of $10 \mu\text{g l}^{-1}$ was initiated without interrupting the infusion of aspirin or indomethacin. On addition of PGE₂ the coronary flow increased but subsided to within control limits (6.43 ± 0.36 ml g⁻¹ min⁻¹) after about 10 min. After 15–20 min of PGE₂ infusion, there was a marked inhibition of the MCD responses (Figs. 1, 2). Again the cardiac hyperactivity induced by tachycardia or by challenging doses of noradrenaline or Ca²⁺ was unaffected by addition of PGE₂. Furthermore, it seems that this inhibitory action is specific for the MCD response, as the vasodilatory effects of adenosine were not affected by aspirin. Increase in coronary flow with adenosine was $7.3 \pm 2.71\%$ and with adenosine plus aspirin $6.8 \pm 1.17\%$ ($P > 0.1$).

It seems that coronary vasodilatation, leading to improved coronary flow, is one of the major adaptative mechanisms of intracardiac origin that permit the coronary circulation to compensate for increased metabolic demands because of increased cardiac activity. As increased levels of cyclic AMP

correlate with the MCD response to cardiac hyperactivity³, it is our belief that cardiac hyperactivity leads to activation of adenylyl cyclase and that this is the triggering mechanism which controls the tone of the coronary vascular smooth muscle of the coronaries. This belief is supported by the observation that if cyclic AMP levels are lowered by inhibiting adenylyl cyclase activation with PGE₂, cardiac hyperactivity is then followed by a reduced MCD response². If phosphodiesterase inhibitors are administered to the rat heart, however, potentiation of MCD and increased levels of cyclic AMP occur^{2,3}.

It has been shown that synthesis of PG may be elicited by noradrenaline administration or by sympathetic nerve stimulation^{15,18} and that this synthesis can also be inhibited by aspirin or indomethacin¹⁹. PG inhibits the release of catecholamines, and it has been postulated that PG plays a role as a feedback braking system which controls the release of noradrenaline from adrenergic nerve endings^{20,21}.

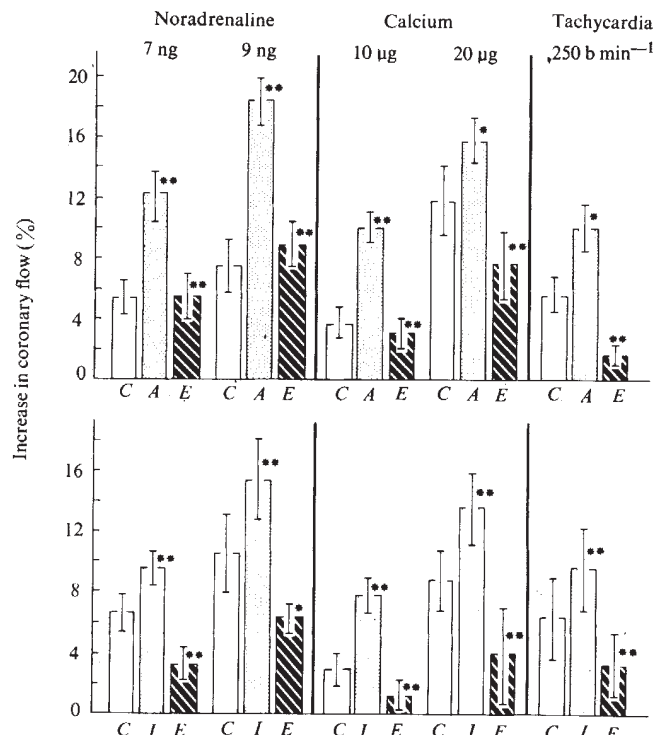


Fig. 2 Changes in metabolic coronary dilation responses due to aspirin (a) and indomethacin (b) before and during PGE₂ infusion. The bars depict increase in coronary flow $\pm$ s.e. over basal levels when the heart is stimulated with noradrenaline, calcium and tachycardia, during control (C), aspirin or indomethacin infusion (A or I) and PGE₂ infusion (E). By analysis of paired data, comparisons were made between responses during the control period, C, and during infusions of aspirin, A, or indomethacin, I, and between the latter, A or I, alone and during PGE₂ infusions, E. *, $P = 0.025-0.01$. **, $P < 0.01$.

We have hypothesized that endogenous PGs are physiological modulators of the metabolic coronary dilation which occurs in response to cardiac hyperactivity, whether induced by noradrenaline or other means. That is, PG mediates a braking system on the adenylyl cyclase activation²². Suppression of PG synthesis therefore results in increased cyclic AMP production and so in the augmented metabolic coronary dilation. The latter cannot be attributed to the release of PG as it is the inhibition of that release which leads to enhanced MCD. Inhibition of PG synthesis does not change the dilation produced by exogenously administered adenosine and so the latter is not likely to be a mediator of the metabolic coronary reaction.

From our concept, we would expect that failure of metabolic coronary dilatation should lead to coronary insufficiency whenever the heart is strained. If this is correct, one may postulate that the administration of an aspirin-like compound or other inhibitor of PG synthesis²⁰ would be useful in preventing coronary insufficiency in conditions of cardiac stress.

We thank L. Oporto and J. Asta for technical assistance. PGE₂ was supplied by Dr J. E. Pike, Upjohn Co., Kalamazoo, Michigan, and indomethacin by Dr W. D. Dorian, Merck, Sharp and Dohme, Canada. This work was supported by the Ontario Heart Foundation.

J. TALESNIK
F. A. SUNAHARA

Department of Pharmacology,
University of Toronto,
Toronto

Received March 12; revised June 1, 1973.

- ¹ Sunahara, F. A., and Talesnik, J., *Proc. Can. Fed. Biol. Soc.*, **14**, 100 (1971).
- ² Sunahara, F. A., Talesnik, J., and Sen, A. K., *Proc. Fifth Int. Cong. Pharmac.*, No. 1350, 225 (San Francisco, 1972).
- ³ Sen, A. K., Sunahara, F. A., and Talesnik, J., *Proc. Fifth Int. Cong. Pharmacol.*, No. 1244, 208 (San Francisco, 1972).
- ⁴ Sunahara, F. A., Sen, A. K., and Talesnik, J., *Can. med. Ass. J.*, **107**, 634 (1972).
- ⁵ Vane, J. R., *Nature new Biol.*, **231**, 232 (1971).
- ⁶ Smith, W. L., and Lands, W. E. M., *J. Biol. Chem.*, **246**, 6700 (1971).
- ⁷ Flower, R., Gryglewski, R., Herbaczynska-Cedro, K., and Vane, J. R., *Nature new Biol.*, **238**, 104 (1972).
- ⁸ Feinberg, H., Boyd, E., and Katz, L. N., *Am. J. Physiol.*, **202**, 643 (1962).
- ⁹ Zachariah, P., *J. Physiol.*, **158**, 59 (1961).
- ¹⁰ Douglas, R. C., Armengol, V., and Talesnik, J., *Acta physiol. latinoam.*, **10**, 205 (1960).
- ¹¹ Hinke, J. A. M., Wilson, M. L., and Burnham, S. C., *Am. J. Physiol.*, **206**, 211 (1964).
- ¹² Somlyo, A. P., and Somlyo, A. V., *Pharmac. Rev.*, **20**, 197 (1968).
- ¹³ Seidel, C. L., and Bohr, D. F., *Circ. Res.*, Suppl. II, **29**, 88 (1971).
- ¹⁴ Gregg, D. E., and Fisher, L. C., in *Handbook of Physiology* (edit. by Hamilton, W. H., and Dow, P.), **2**, 1517 (Williams and Wilkins, Baltimore, 1963).
- ¹⁵ Ferreira, S. H., Moncada, S., and Vane, J. R., *Nature new Biol.*, **231**, 237 (1971).
- ¹⁶ Ackenfels, A., and Vane, J. R., *Br. J. Pharmac.*, **45**, 451 (1972).
- ¹⁷ Tomlinson, R. V., Ringold, H. J., Qureshi, M. C., and Forchielli, E., *Biochem. biophys. Res. Commun.*, **46**, 552 (1972).
- ¹⁸ Wennmalm, Å., and Stjärne, L., *Life Sci.*, **10**, 471 (1971).
- ¹⁹ Junstad, M., and Wennmalm, Å., *Acta physiol. scand.*, **85**, 573 (1972).
- ²⁰ Hedqvist, P., Stjärne, L., and Wennmalm, Å., *Acta physiol. scand.*, **83**, 430 (1971).
- ²¹ Samuelsson, B., and Wennmalm, Å., *Acta physiol. scand.*, **83**, 163 (1971).
- ²² Shio, H., Shaw, J., and Ramwell, P., *Ann. NY Acad. Sci.*, **185**, 327 (1971).

Chemical and Mechanical Requirements for Fibroblast Adhesion

HERE I show that fibroblasts do not adhere to a rigid hydrophobic or yielding hydrophilic surface. There are three aspects; clean surfaces, the smallness of the adhesion sites, and mechanical stresses exerted by the cell.

Surfaces are listed in Table 1 according to wettability¹. Cells spread fully on glass and oxidized polystyrene, less readily² on polyester, and only patchily on, for example, native polystyrene and polyethylene. It was concluded¹ from a discrepancy in surface energies between polyethylene and pure paraffin that "even the best specimens of polyethylene have traces of polar, strongly water-attracting

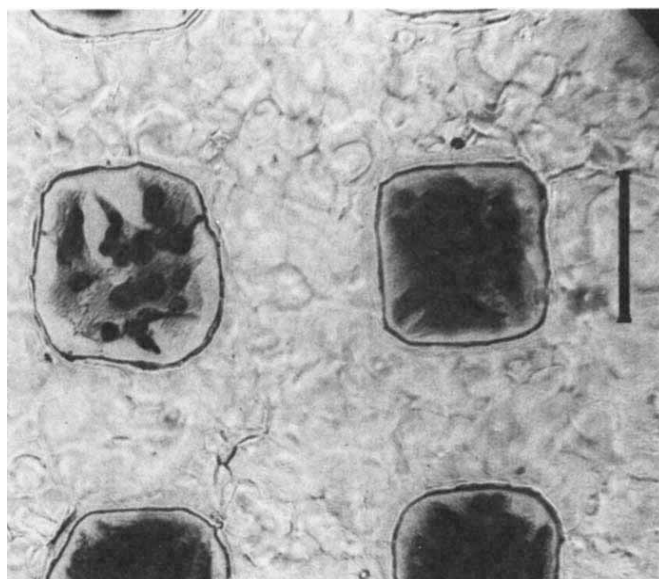


Fig. 1 "Haptotactic islands" of tissue culture surface, with spread cells surrounded by a non-adhesive surface of solid paraffin. Bar=100 μ m.

material present in or near their surfaces". These polar heterogeneities, if small and dispersed, may remain undetected by the drop method for measuring surface energy^{1,3} because the drop, mm in diameter, is a less localized probe than the cell which makes adhesive "plaques" of only 1 μ m⁴.

Hypothetically, cells should not be able to spread on a surface of solid paraffin, free from polar impurities. Paraffin waxes were dissolved at 5% v/v in hexane, and polar material was extracted by shaking with Al₂O₃ (BDH Ltd) before use; 0.1 ml was dried on a 3 cm polystyrene tissue-culture dish. Cells were added (10⁵ BHK or 3T3, in 2 ml medium with 10% calf serum) and incubated for 3 days at 37° C. Paraffin was found to be non-adhesive for mouse and hamster fibroblasts.

Table 1 Surface Energies of Some Materials in Tissue Culture¹

Material	Surface energy (dyne cm ⁻¹)
Fluoropolymer	19
Paraffin (—CH ₃)	23
Polyethylene	31
Polystyrene (native)	33
Polyester	43
Polystyrene (corona-treated)	56
Water	72
Glass	About 700

Samples with melting point 55–65° C were satisfactory, but those with a melting point of 49° C softened in the incubator, and retracted from the hydrophilic tissue culture surface. This suggested a convenient method of preparing "haptotactic islands"¹⁵ for dividing the growth surface into shaped colonies. An electron microscope grid (Smethurst Ltd, Bolton) was dipped into paraffin solution, and laid in a tissue culture dish at 50–60° C. The paraffin melted and retracted from the holes of the grid, but remained in position under the surrounding metal surface. After cooling the grid was peeled away, leaving its paraffin replica outlining hydrophilic "islands" on the dish (Fig. 1). The film can be scratched to expose the underlying tissue culture surface; cells will attach and migrate in such a track.

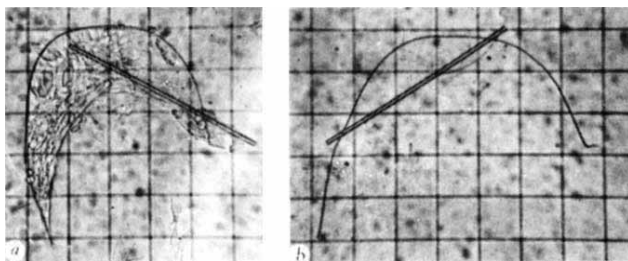


Fig. 2 Demonstration that fibroblasts exert tension on their substrate. BHK C13 cells were mixed with fine glass fibres (Whatman GF100) and grown in drops of medium, under paraffin oil, on a bacteriological polystyrene dish. Cells were lysed by adding a drop of detergent (SDS in 0.2 N NaOH). *a*, Curved fibre, spanned by a web of cells; *b*, same fibre, after lysis of cells. Scale: 1 division = 100 μ m.

Agar is hydrophilic. Why do cells not normally spread on it^{6,7} while they will extend both on and in a gel of dilute collagen^{6,8}? Agarose molecules form an interlacing network of hydrogen-bonded double helices, with periodic "kinks" in each molecule, which prevent complete pairing between any two strands of the helix, ensuring that they will branch off and form new helices with at least one other partner⁹. This intercoiled molecular structure leads to a gel which is macroscopically solid, resilient, and precise in shape, but which, on the cellular scale, may be a diffuse "ethereal haze"¹⁰, unable to support a concentrated load. Collagen molecules associate in long, 3 $\times$ 5 stranded helices, side by side in dense, straight filaments¹¹, 0.01–0.1 μ m thick. Macroscopically, collagen gels are liable to collapse⁸ but, on the cellular scale, the fibres offer localized support. (Thus a gel of collagen fibres in agar would be a crude model of connective tissue: in cartilage, collagen provides strength and rigidity, while mucopolysaccharides govern resilience and permeability¹².) The electron microscopy of agar, collagen and the cell coat, and their mechanical interactions, have been discussed^{13,14}.

We have found¹⁵ that isolated fibroblasts in agar, if they are allowed to stretch in one dimension, will grow on fine glass fibres (Whatman GF100), 0.05–0.5 μ m thick. The thinner fibres of glass approach collagen dimensions; that fibroblasts will stretch on them is further indication of the localized extent of cell adhesion to substrates. The bowed shape of some fibres suggests that the cells exert tension because the bows straighten if the "strings" are lysed (Fig. 2).

I postulate that fibroblasts require a rigid scaffolding to withstand tension generated by their stretching. Glass, or the fibres in a collagen gel, even if much thinner than the cell, can provide such a scaffolding; more diffuse gels cannot. (Cells will spread, although imperfectly, on dried agar¹⁶.)

The observation that cells do not spread on a film of calf-brain phospholipid¹⁷ can be explained. Membrane lipids are fluid and orientate with hydrophilic groups towards water. Mechanically, multilayers of calcium stearate resist normal load, but break up under transverse load, so that sliding occurs within a mush of stearate¹³. Phospholipid films therefore may provide another example of a hydrophilic surface of low yield strength.

Experimentally, egg lecithin (BDH) was found to form a non-adhesive substrate, applied as 0.1–0.2 ml of a 1% solution in hexane. Unlike paraffin, this phospholipid surface was readily wetted. When scratched in medium containing serum at 37° C, the lecithin layer displayed, under the microscope, the consistency of a thixotropic, viscoelastic fluid. Cells did not attach to the layer, but readily grew in tracks scratched through it.

It might be supposed that, if cells do not adhere to a hydrophobic surface, and do not spread on a yielding surface, then the least adherent interface would be that of

a hydrophobic fluid. Cells grow, however, on the interface between medium and droplets of silicone oil. The solution to this paradox is found by probing the interface with a finely drawn glass fibre under the microscope. The surface of the oil drop puckers, and the fibre bends. A skin can be seen which consists of denatured serum proteins, which, with hydrophobic interiors now exposed to the oil, bridge the great gap in surface tension (40–50 dyne cm⁻¹) between oil and water. Such skins accumulate readily, even at the interface between purified liquids, withstand considerable shear forces¹⁸, and provide a rigid hydrophilic surface on which cells can spread. Conversely, the amphipathic, neutral, lecithin surface shows little tendency to adsorb and denature proteins¹⁹, producing the mobility of the phospholipid interface, compared to that of an oil, in serum.

That the cell's attachment sites seem to be hydrophilic and small agrees with the detection of dense ruthenium-red staining (that is, acid polysaccharide) material at plaques, both *in vitro* (S. Pegrum, personal communication) and *in vivo*¹⁰. As for exerting tension, microfilaments, possibly contractile, have been observed in the cytoplasm, pointing towards attachment plaques near the leading edge of the cell⁴.

The alkane surface, with its CH₃- and CH₂-groups, is a chemically defined base line; by systematic introduction of other components, it should be possible to obtain a more detailed picture of the chemical bonds involved in cell adhesion. Because the surface is rigid, one can vary not only molecular concentration, but also spatial distribution. Clustering may be important for cells to make strong, localized bonds.

It is notable that, of the two non-adhesive, hydrophilic substrates, agarose is uncharged and phosphatidyl choline is an almost neutral zwitterion²⁰. Adhesiveness, even in the absence of like charges, can be decreased by the exclusion volume²¹ whereby one randomly coiling molecule tends to oust another: this effect might explain reduced adhesion in serum¹⁸.

N. G. MAROUDAS

Imperial Cancer Research Fund,
PO Box 123, Lincoln's Inn Fields,
London WC2A 3PX

Received April 19; revised May 14, 1973.

- Gould, R. F. (ed.), *Contact Angle, Wettability and Adhesion*, Adv. Chem. Ser., 43 (Amer. Chem. Soc., 1964).
- House, W., Shearer, M., and Maroudas, N. G., *Expl. Cell Res.*, **71**, 293 (1972).
- Schönhorn, H., in *Progress in Membrane and Surface Science* (edit. by Danielli, J. F., Rosenberg, M. D., and Cadenhead, D. A.), **5** (Academic, London, New York, 1972).
- Abercrombie, M., Heaysman, J. A. M., and Pegrum, S. P., *Expl. Cell Res.*, **67**, 359 (1971).
- Carter, S. B., *Nature*, **208**, 1183 (1965).
- Lieberman, M., Roggeven, A. E., Purdy, I. E., and Johnson, E. A., *Science*, **N.Y.**, **175**, 909 (1972).
- Maroudas, N. G., *Expl. Cell Res.*, **74**, 337 (1972).
- Elsdale, T., and Bard, J., *J. cell Biol.*, **54**, 626 (1972).
- Dea, I. C. M., McKinnon, A. A., and Rees, D. A., *J. molec. Biol.*, **68**, 153 (1972).
- Luft, J. H., *Anat. Res.*, **171**, 369 (1971).
- Miller, A., and Parry, D. A. D., *J. molec. Biol.*, **75**, 441 (1973).
- Maroudas, A., in *Adult Human Articular Cartilage* (edit. by Freeman, M. A. R.) (Pitman, in the press).
- Maroudas, N. G., and Sawistowski, H., *Chem. Eng. Sci.*, **19**, 919 (1964).
- Israelachvili, J. N., and Tabor, D., *Nature*, **241**, 148 (1973).
- Maroudas, N. G., *Expl. Cell Res.* (in the press).
- Abercrombie, M., in *Cell, Tissue and Organ Culture*, Monograph No. 26, National Cancer Institute, Bethesda (1967).
- Ivanovna, O. Y., and Margolis, L. B., *Nature*, **242**, 200 (1973).
- Taylor, A. C., *Expl. Cell Res., Suppl.*, **8**, 154 (1961).
- Dawson, R. M. C., in *Biological Membranes* (edit. by Chapman, D.), I (Academic Press, London, New York, 1968).
- Seimiya, T., and Ohki, S., *Biochim. Biophys. Acta*, **298**, 546 (1973).
- Hesseling, F. T., Vrij, A., and Overbeek, J. T. G., *J. phys. Chem.*, **75**, 2094 (1971).

Evidence that Cyclic AMP Does Not Mediate the Action of Gibberellic Acid

MANY animal hormones operate by increasing the intracellular level of cyclic AMP, which then acts as a second messenger to elicit a variety of physiological effects within the cell¹. There is no conclusive evidence that cyclic AMP performs a similar function in higher plants. Exogenously supplied cyclic AMP can mimic certain effects of plant hormones²⁻⁴, but the responses do not equal the full effect of the hormone itself and may be pharmacological. Reports that plant hormones can elevate cyclic AMP levels in plant tissues^{5,6} lack sufficiently rigorous proof that the factor being measured is indeed the cyclic nucleotide. The criteria of identity most frequently employed, selective precipitation⁷ and cochromatography, are inadequate to resolve pmol quantities of cyclic AMP from nmol or μ mol of other adenine nucleotides. I have investigated the possibility that cyclic AMP is synthesized in response to gibberellic acid (GA) in barley aleurone layers. I have measured the incorporation of ¹⁴C-adenosine into cyclic AMP in barley aleurone layers, and my results do not support the suggestion that the cyclic nucleotide mediates the action of GA^{2,3,5}.

GA induces the synthesis of α -amylase in isolated barley aleurone layers, the enzyme first appearing after a lag phase of 6 to 8 h (ref. 8). GA at 10^{-6} M accelerates the synthesis of α -amylase some thirty-fold. An increase in the level of cyclic AMP should precede the GA response if cyclic AMP mediates that response⁹. To demonstrate any effect on cyclic AMP synthesis during GA treatment, the hormone must be applied at a time when endogenous ATP is adequately labelled by applied ¹⁴C-adenosine. In barley aleurone layers, labelled adenosine (94 nmol per 100 layers) equilibrates into endogenous ATP within 12 h of incubation (Fig. 1). In the experiments below, I used a uniform incubation period of 12 h with U-¹⁴C-adenosine (50 μ Ci; 94 nmol per 100 layers) at high specific activity (534 mCi mmol⁻¹, Amersham-Searle). GA was added for various periods from 0 to 8 h before termination of incubation.

Cyclic AMP was isolated by grinding aleurone layers at 0° C in 10% trichloroacetic acid (TCA) containing 8-³H-cyclic AMP as internal standard (approximately 240,000 c.p.m. per 100 aleurone layers). Debris and precipitated protein were removed by centrifugation and protein in the pellet was estimated by the method of Lowry¹⁰. TCA was extracted from the supernatant by five successive washes with diethyl ether (four volumes). The sample was applied to a column of DEAE-'Sephadex' A25 (24 $\times$ 1.5 cm). A non-linear gradient was used for elution of adenine nucleotides. The gradient was formed in three equal chambers (500 ml each) containing respectively 0.02 M NH₄HCO₂, pH 4.8; 0.2 M NH₄HCO₂, pH 4.8; and 0.4 M NH₄HCO₂, pH 8.6. Fractions containing cyclic AMP were identified by the tritiated internal standard (Fig. 2). ATP was the major radioactive component eluted (60% of incorporated ¹⁴C). The specific radioactivity of ATP was measured to

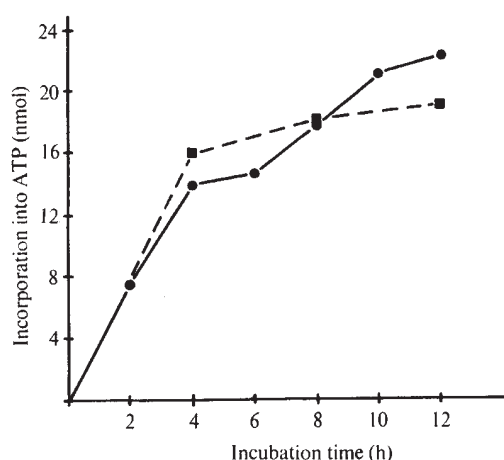


Fig. 1 Incorporation of adenosine into ATP by barley aleurone layers. Aleurone layers were isolated from barley seed (*Hordeum vulgare* L. cv. Himalaya) by the method of Chrispeels and Varner⁸. Sample layers were tested for sterility by incubation on nutrient agar. 100 layers were incubated at 25° C, with shaking, in groups of twenty layers per 50 ml Erlenmeyer flask containing 4 ml medium (1 mM NaOAc, pH 4.8, 20 mM CaCl₂). GA solution was sterilized by 'Millipore' filtration and was added to a final concentration of 10^{-6} M when appropriate. For determination of incorporation into ATP, 8-³H-adenosine (15×10^6 c.p.m., 18.8 nmol) was included in each flask. Adenine nucleotides were isolated by grinding aleurone layers in ice cold 10% TCA, and analysed by ion-exchange chromatography. (●) Without GA, (■) with GA.

compensate for twenty-five to thirty-fold dilution of labelled adenosine by endogenous pools of adenine nucleotides. The recovery of ATP and the ratio of ATP to ADP (12.5:1) indicated that breakdown of nucleotide phosphates during isolation had been minimal. The cyclic AMP recovered from the ion-exchange column was chromatographed twice on cellulose thin layers (0.5 mm) using first isopropanol and 0.03 M NH₄HCO₃, pH 8.6 (3:1 v/v), and then isopropanol, 0.1 M H₃BO₃ and saturated NH₄OH (7:2:1 v/v). Table 1 shows the recovery of ³H and ¹⁴C during the purification of cyclic AMP.

There is considerable enrichment of ³H-internal standard with respect to incorporated ¹⁴C at each stage of purification. This differential recovery of isotopes was not observed when authentic ¹⁴C-cyclic AMP cochromatographed with ³H-cyclic AMP. Clearly, most of the ¹⁴C recovered at earlier stages during purification is not in the form of cyclic AMP.

The cyclic AMP from the second thin layer chromatography (TLC) step was recovered from scintillation fluid by adsorption on Whatman 3 MM paper (50 to 70% recovery). Paper electrophoresis was used as a fourth stage of purification (2.5 h at 20 V cm⁻¹ in 0.06 M NH₄OAc, pH 3.5). Enrichment of ³H-cyclic AMP with respect to ¹⁴C was again observed (Table 2). It is doubtful therefore that the remaining ¹⁴C recovered

Table 1 Incorporation of U-¹⁴C-Adenosine and Recovery of Cyclic AMP in Barley Aleurone Layers

	0	0.5	Duration of GA treatment (h)				8	No GA
			1	2	4			
¹⁴ C-adenosine in medium ($\times 10^{-6}$)	104.6	104.0	103.2	105.3	103.5	100.0		96.1
¹⁴ C-uptake ($\times 10^{-6}$)	35.3	36.0	34.9	32.6	32.8	34.4		31.6
³ H-cyclic AMP added as standard	237,345	493,905	490,470	237,525	226,500	221,535		232,635
Cyclic AMP from ion-exchange	{	{	{	{	{	{	{	{
	³ H	112,690	219,150	226,512	116,980	109,280	129,878	124,070
	¹⁴ C	25,880	24,690	25,587	31,500	29,240	28,056	24,130
Cyclic AMP from first TLC	{	{	{	{	{	{	{	{
	³ H	109,540	155,022	216,636	80,206	83,614	95,076	104,556
	¹⁴ C	4,910	3,360	1,460	2,296	2,848	392	2,012
Cyclic AMP from second TLC	{	{	{	{	{	{	{	{
	³ H	75,011	106,212	126,040	71,241	78,504	101,040	98,649
	¹⁴ C	41.8	84.5	90.6	35.3	48.6	67.9	44.6

All incubations were for a total of 12 h, otherwise conditions were as for Fig. 1. GA was added for the periods indicated before termination of the incubation. All values are c.p.m.

Table 2 Recovery of Cyclic AMP after Electrophoresis, and Estimate of Possible Cyclic AMP Synthesis during the Incubation

	0	0.5	Duration of GA treatment (h)				8	No GA
			1	2	4			
³ H-cyclic AMP added as standard (c.p.m.)	237,345	493,905	490,470	237,525	226,500	221,535	232,635	
Cyclic AMP from electrophoresis { ³ H (c.p.m.) { ¹⁴ C*	43,317	53,010	81,782	28,963	30,227	40,597	39,672	
Recovery (%)	12.4	5.9	16.1	7.2	7.8	11.5	9.7	
Total ¹⁴ C in putative cyclic AMP (c.p.m.)	18.2	10.7	16.7	12.2	13.4	18.3	17.1	
Specific radioactivity of isolated ATP (c.p.m. pmol ⁻¹)	67.9	54.6	95.6	58.8	58.1	62.6	56.9	
Estimate of ¹⁴ C as cyclic AMP (pmol)	32.1	33.0	27.5	30.7	29.3	34.8	26.2	
	2.1	1.65	3.5	1.9	2.0	1.8	2.2	

* Based on counts accumulated in 60 min, and after subtraction of 10.2 c.p.m. background. Additional background counts that did not originate from the adsorbed sample were eluted from electrophoresis paper. The variability of contamination prevented estimation of individual errors, and the results reported remain uncorrected. 3 to 6 c.p.m. of the ¹⁴C counts recovered after electrophoresis may originate by contamination from this source. Eluates from unused electrophoresis paper contained similar high background counts.

with cyclic AMP really represents incorporation of labelled precursor into the cyclic nucleotide. Losses incurred, coupled with high background counts, prevented measurement of ¹⁴C retention with the ³H-cyclic AMP standard after further purification.

With the above data, synthesis of cyclic AMP in barley aleurone layers seems unlikely, but cannot be disproved beyond the detection limits of this procedure. The estimate of 2 to 3 pmol per 100 aleurone layers (1.5 g fresh weight or 75 mg protein) is best regarded as an upper limit for the possible presence of the cyclic nucleotide, a concentration one hundredth of that found in animal cells without hormonal stimulation^{12,13}. Also there is no sustained elevation of cyclic AMP synthesis resulting from treatment at a GA concentration (10⁻⁶ M) that induces the maximum amount of α -amylase⁸. It seems unlikely that transient increases in cyclic AMP concentration could play a role in GA action, because the hormone must be continuously present to maintain α -amylase synthesis. With GA at 10⁻⁶ M, an intracellular concentration of cyclic AMP no greater than 10⁻⁹ M would be inconsistent with the concept of the cyclic nucleotide operating as an amplification system for hormones¹⁴. That cyclic AMP mediates the effects of GA in barley aleurone layers^{3,5} must therefore be seriously doubted.

Other evidence also suggests that analogies between the possible functions of cyclic nucleotides in higher plants and the system operating in animal cells are not reliable. Cyclic nucleotide phosphodiesterase from pea seedlings¹⁵ differs from

the corresponding enzyme in animal cells¹⁶ in its inability to discriminate between 2',3'- and 3',5'-cyclic nucleotides, by its product of hydrolysis, 3'-nucleotide, and by the failure of methylxanthines to inhibit its activity. A similar enzyme is found in barley (P. P. C. Lin, personal communication). My own work has shown that papaverine and the methylxanthines, theophylline and caffeine, do not retard the degradation of exogenously supplied ³H-cyclic AMP by barley aleurone layers (unpublished observations). Inclusion of papaverine, theophylline or caffeine (each at 5 mM) in 12 h incubations of aleurone layers with ¹⁴C-adenosine does not raise the recovery of ¹⁴C with ³H-cyclic AMP internal standard, using the procedures described here. Protein kinases activated by cyclic nucleotides are widely distributed in animal tissues¹⁷. The regulatory properties of cyclic nucleotides seem to result from their control of the phosphorylation of critical cellular proteins in such tissues¹⁸. Protein kinases isolated from plant tissues are not activated *in vitro* by cyclic nucleotides (in preparation). Cyclic AMP is present in yeast¹⁹, but is inactive on yeast protein kinase, and cyclic AMP-binding protein isolated from yeast has no protein kinase activity²⁰. Cyclic AMP levels in yeast are raised by glucose stress¹⁹, a response similar to that in *Escherichia coli*²¹. It seems likely that the role of the cyclic nucleotides in higher plants, if they do occur in these tissues, is quite distinct from their known functions in animal cells.

This work was supported by the US Atomic Energy Commission and was completed during tenure of a NATO post-doctoral fellowship from the Science Research Council, London.

ROBERT A. B. KEATES

MSU/AEC Plant Research Laboratory,
Michigan State University,
East Lansing, Michigan 48823

Received April 13, 1973.

- Sutherland, E. W., *Science*, **N.Y.**, **177**, 401 (1972).
- Duffus, C. M., and Duffus, J. H., *Experientia*, **25**, 581 (1969).
- Galsky, A. G., and Lippincott, J. A., *Pl. Cell Physiol.*, **Tokyo**, **10**, 607 (1969).
- Kamisaka, S., Sakurai, N., and Masuda, Y., *Pl. Cell Physiol.*, **Tokyo**, **14**, 183 (1973).
- Pollard, C. J., *Biochim. biophys. Acta*, **201**, 511 (1970).
- Azhar, S., and Krishna Murti, C. R., *Biochem. biophys. Res. Commun.*, **43**, 58 (1971).
- Krishna, G., Weiss, B., and Brodie, B. B., *J. Pharmac. exp. Ther.*, **163**, 379 (1968).
- Chrispeels, M. J., and Varner, J. E., *Pl. Physiol.*, **Lancaster**, **42**, 398 (1967).
- Sutherland, E. W., in *Cyclic AMP* (edit. by Robison, G. A., Butcher, R. W., and Sutherland, E. W.), 36 (Academic Press, New York, 1971).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
- Bock, R. M., Ling, N. S., Morell, S. A., and Lipton, S. H., *Archs. Biochem. Biophys.*, **62**, 253 (1956).
- Kuo, J. F., and Greengard, P., in *Advances in Cyclic Nucleotide Research* (edit. by Greengard, P., and Robison, G. A.), **2**, 41 (Raven Press, New York, 1972).

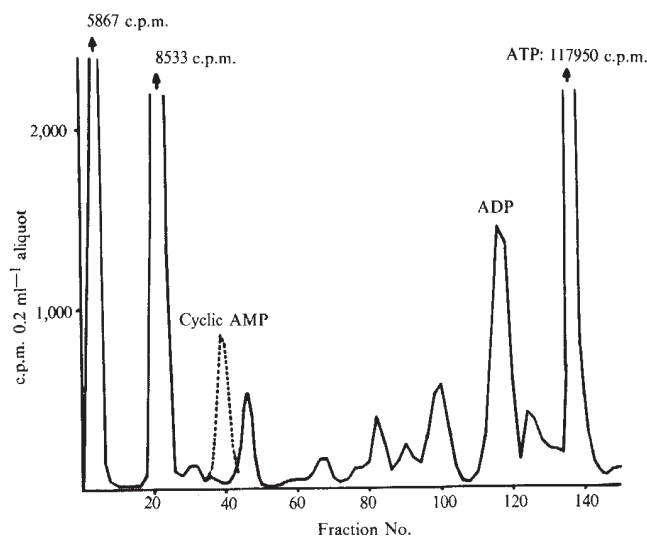


Fig. 2 Elution of adenine nucleotides from DEAE-Sephadex A25. Radioactivity was determined in 0.2 ml aliquots from each 10 ml fraction, by liquid scintillation counting. ATP was estimated by absorbance measurements at 260 and 290 nm (ref. 11) on the peak fraction (fraction No. 137 in this example). (—) ¹⁴C, (---) ³H.

- ¹³ Steiner, A. L., Pagliara, A. S., Chase, L. R., and Kipnis, D. M., *J. biol. Chem.*, **247**, 1114 (1972).
- ¹⁴ Bowness, J. M., *Science*, **152**, 1370 (1966).
- ¹⁵ Lin, P. P. C., and Varner, J. E., *Biochim. biophys. Acta*, **276**, 454 (1972).
- ¹⁶ Butcher, R. W., and Sutherland, E. W., *J. biol. Chem.*, **237**, 1244 (1962).
- ¹⁷ Kuo, J. F., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1349 (1969).
- ¹⁸ Krebs, E. G., in *Current Topics in Cellular Regulation* (edit. by Horecker, B. L., and Stadtman, E. R.), **5**, 99 (Academic Press, New York, 1972).
- ¹⁹ Sy, J., and Richter, D., *Biochemistry*, **11**, 2788 (1972).
- ²⁰ Sy, J., and Richter, D., *Biochemistry*, **11**, 2784 (1972).
- ²¹ Pastan, I., and Perlman, R. S., *Science*, **169**, 339 (1970).

Vitamin D-like Action of *Solanum malacoxylon* on Calcium Transport by Rat Intestine

A DISEASE of cattle, presenting gross symptoms of calcification of heart, lungs and other tissues, and known as *enteque seco* in Argentina or as *espichamento* in Brazil, has been shown to be due to the animals grazing a shrub, *Solanum malacoxylon*¹. Oral administration of the dried leaf of the plant can reproduce the symptoms of the disorder in cattle². In a study of calcium balance in cattle it was shown that oral dosing with the ground leaf could significantly reverse a negative calcium balance within a few days; the same workers concluded by use of a whole-body counting technique and tracer isotope that the increased retention of calcium was due to greater absorption rather than diminished calcium loss³. The similarity of the clinical symptoms to vitamin D intoxication, taken along with these findings, suggests that the plant leaf may exert an effect on calcium absorption similar to that of vitamin D. We report here a remarkable similarity in action of oral dosing of rats with *S. malacoxylon* leaf and of vitamin D administration on the initial rates of uptake of calcium by intestinal mucosal preparations.

The experiments were made on both normal and vitamin D-deficient animals. Albino rats were weaned at about 50 g to either a normal (Oxoid 41B) or a modified Steenbock rachitogenic diet (Ca 1%, P 0.25%). At 3 weeks after weaning the normal animals had a mean weight of 140 g and the D-deficient animals 100 g. On both the third and second days before experiment, four rats from each group were given orally 100–150 mg of ground leaf suspended in water. Another four animals from each group were each given 50,000 IU of cholecalciferol (vitamin D₃) intraperitoneally 48 h before the experiment. Three untreated control animals completed each dietary group. Food but not water was withheld for 24 h from all animals before starting the experiments.

The animals were decapitated and a segment of intestine, extending 6 cm distal to the pylorus, rapidly removed and mounted for measurement of the initial kinetics of calcium uptake across the mucosal surface, as has been described elsewhere⁴. Conditions of incubation of the tissue were the same for all twenty-two rats, and the procedure is given in summary with Table 1. A calcium concentration of 1.0 mM was used, as in these conditions significant effects of vitamin D on initial kinetics of calcium uptake have been demonstrated⁵.

Rats which had been on a normal diet responded to treatment with vitamin D (Table 1), the mean uptake being greater than in untreated controls at all three times of incubation. The rates of Ca²⁺ uptake calculated were 1.11 ± 0.22 and 1.93 ± 0.23 nmol cm⁻² min⁻¹ for control and treated rats, respectively (means $\pm$ s.e.). The dose of vitamin administered was large and this change in rate of Ca²⁺ uptake can be regarded as a maximum effect for this group of rats. For similar rats given *S. malacoxylon* leaf, the uptakes at 3 and 5 min were also significantly higher; at 1 min the value was higher than

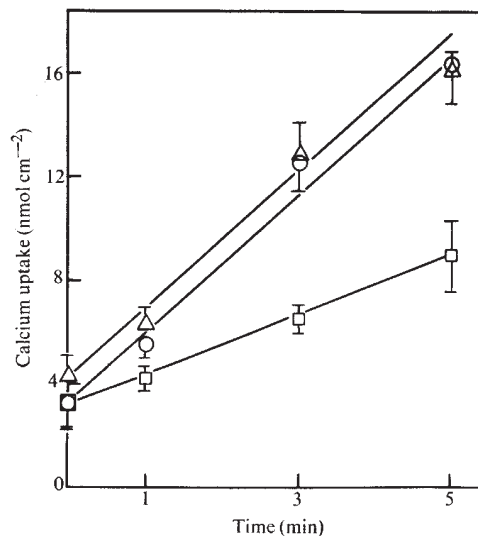


Fig. 1 Calcium uptake by the small intestine of rats raised on a diet deficient in vitamin D. Conditions of the experiment are as described in Table 1. □, Control rats; ○, rats given 50,000 IU vitamin D₃ 48 h before experiment; △, rats given 100 mg of powdered *S. malacoxylon* leaf 72 and 48 h before the experiment. Points give mean values $\pm$ s.e. of from six to twelve determinations; lines are generated assuming uptake to be linear with time from 1–5 min incubation.

in the controls but this was not statistically significant. Perhaps reflecting the smaller differences at each time of incubation, the rate of uptake in the *S. malacoxylon*-treated animals was somewhat less than after vitamin D, but at 1.70 ± 0.27 nmol cm⁻² min⁻¹ this was still substantially greater than for the control.

Table 1 Calcium Uptake by Intestinal Mucosa from Normal and Vitamin D-deficient Rats Treated with Vitamin D₃ or *Solanum malacoxylon* Leaf

Diet	Incubation (min)		
	1	2	3
Normal			
Control	3.33 $\pm$ 0.33	5.85 $\pm$ 0.60	7.78 $\pm$ 0.91
D ₃	5.17 $\pm$ 0.27 *	11.17 $\pm$ 0.58 *	12.89 $\pm$ 0.85 *
<i>S. malacoxylon</i>	4.64 $\pm$ 0.35	9.24 $\pm$ 0.56 †	11.37 $\pm$ 1.01 ‡
D-deficient			
Control	4.20 $\pm$ 0.59	6.50 $\pm$ 0.53	9.02 $\pm$ 1.39
D ₃	5.55 $\pm$ 0.53	12.51 $\pm$ 1.17 *	16.33 $\pm$ 1.56 *
<i>S. malacoxylon</i>	6.34 $\pm$ 0.61 †	13.00 $\pm$ 1.21 *	16.20 $\pm$ 0.68 *

Numbers are mean calcium uptakes ($\pm$ s.e. for six to twelve replicates) expressed as nmol Ca²⁺ cm⁻² mucosal surface. Statistical significance of difference from appropriate control, $P < 0.001$ *, $P < 0.01$ †, $P < 0.05$ ‡. Segments of intestine were cut longitudinally and mounted so that the mucosal surface was bathed by phosphate-free, Tris-buffered physiological saline, oxygenated, at 37° C and pH 7.4. So mounted, a segment from one rat provided eight replicate areas of tissue each of 0.17 cm² and times of incubation were randomly distributed within each segment. Calcium uptake was determined as ⁴⁵Ca activity in the tissue after incubation with medium containing a trace of ⁴⁵CaCl₂. Influx of Ca was terminated by a rapid wash with ice-cold, isosmotic mannitol. The incubated tissue was stamped out of the intestinal strip, leached in dilute nitric acid and ⁴⁵Ca counted by liquid scintillation spectrometry. A trace of ³H labelled inulin was also in the medium and the tissue activity associated with inulin was deducted from the total tissue ⁴⁵Ca activity in order to compute calcium uptake into mucosal cells.

Results for rats kept on a vitamin D-deficient diet are also shown in Table 1. Here the parallel between vitamin treatment and *S. malacoxylon* treatment is striking. At both 3 and 5 min incubation, highly significant increases, of similar magnitude, were obtained in both groups compared with controls. The corresponding regression lines, calculated assuming uptake to be linear from 1 to 5 min incubation, are shown in Fig. 1. The rate of uptake for vitamin D- and *S. malacoxylon*-treated rat intestine was not different (2.70 ± 0.26 nmol Ca²⁺ cm⁻² min⁻¹ and 2.47 ± 0.24 respectively) and for control animals uptake

was less than half this value ($1.14 \pm 0.33 \text{ nmol cm}^{-2} \text{ min}^{-1}$). All regression lines gave positive intercepts on the ordinate. This has been a consistent finding and has been attributed previously to a rapid initial uptake of Ca^{2+} , probably by non-specific adsorption to the microvillar surface^{4,5}. Treatment with vitamin D or *S. malacoxylon* did not affect this rapid phase of Ca^{2+} uptake (3.22 ± 0.91 and $4.31 \pm 0.83 \text{ nmol cm}^{-2}$ for intestines taken from vitamin D- and *S. malacoxylon*-treated rats compared with $3.27 \pm 1.15 \text{ nmol cm}^{-2}$ for controls).

Feeding the ground leaf of the plant can therefore elicit, within the same time, effects similar to vitamin D in normal rats and the effects of both vitamin D and leaf become more pronounced with rats kept on a rachitogenic diet. Cholecalciferol (vitamin D₃) is known to require two successive hydroxylations, occurring in the liver⁶ and kidney cortex⁷, to yield the active metabolite, 1,25-dihydroxycholecalciferol, and it may be that the active principle of the plant has also to be metabolized before it produces its final effect. Unlike the precursors of vitamin D, however, the active principle of *S. malacoxylon* leaf is water-soluble¹ and this would seem to preclude its inclusion with the oil-soluble vitamins. This water solubility might explain the ready extraction of the active material in the gut of different species, but the identity and mode of action remain unknown. In this preliminary investigation, no attempt was made to determine an optimum dose or route of administration for the leaf. Post-mortem examination showed clear traces of unabsorbed leaf material in the colon and one could assume, from the effects produced on Ca^{2+} absorption in the small intestine, that the amount of leaf administered was more than sufficient to elicit a maximum response.

The present unequivocal demonstration that this particular species of solanaceous plant contains one or more active principles which closely resemble cholecalciferol in stimulating calcium absorption suggests a chemical similarity with known precursors of this vitamin, while its water solubility tends to deny such a relationship. This apparent paradox can only be solved by the chemical identification of the active principle which should prove interesting as it might eventually find a clinical use and could give new insight into the general mechanism by which these types of compounds change calcium metabolism.

We thank Dr B. F. Sansom for his gift of powdered *S. malacoxylon* leaf.

J. M. O'DONNELL
M. W. SMITH

ARC Institute of Animal Physiology,
Babraham, Cambridge

Received April 30, 1973.

¹ Worker, N. A., and Carrillo, B. J., *Nature*, **215**, 72 (1967).

² Döbereiner, J., Tokarnia, C. H., da Costa, J. B. D., and Dayrell, M. de S., *Pesquisa Agropecuária Brasileiro, Série Veterinária*, **6**, 91 (1971).

³ Sansom, B. F., Vagg, M. J., and Döbereiner, J., *Res. vet. Sci.*, **12**, 604 (1971).

⁴ O'Donnell, J. M., and Smith, M. W., *J. Physiol., Lond.*, **229**, 733 (1973).

⁵ O'Donnell, J. M., and Smith, M. W., *Biochem. J.* (in the press).

⁶ Horsting, M., and DeLuca, H. F., *Biochem. biophys. Res. Commun.*, **36**, 251 (1969).

⁷ Lawson, D. E. M., Fraser, D. R., Kodicek, E., Morris, H. R., and Williams, D. H., *Nature*, **230**, 228 (1971).

Mechanism for the Chromosome Banding Phenomenon

MAMMALIAN constitutive heterochromatin probably occurs in a variety of forms but chiefly two modes¹. These are (a) centromeric, which is found chiefly at the centromeres and paracentric constrictions, and (b) intercalary, which is found in other chromosomal regions. Our experience with the banding

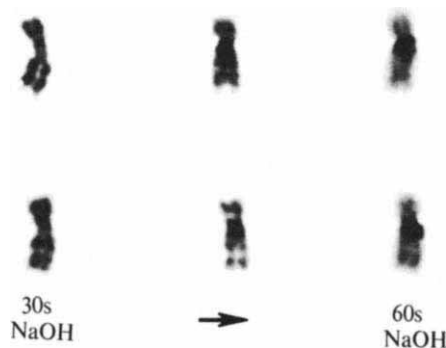


Fig. 1 Human No. 9. Chromosome pairs from different metaphases subjected to different NaOH treatments. The centre pair are G-C band hybrids. Staining time with Giemsa is 3 min with 30 s NaOH treatment and 25 min with 60 s NaOH treatment.

techniques has led us to believe that the G bands (so called by a convention adopted by the Fourth Congress of Genetics, Paris, 1971) contain a considerable complement of intercalary constitutive heterochromatin. We propose that it is chiefly this latter type of A-T rich constitutive heterochromatin that is enriched at the sites of the G bands, and that in the G banding techniques Giemsa stains the protein remaining at these sites after the various pretreatments.

Reproducing the C band (by convention from the Fourth Congress of Genetics) technique of Arrighi and Hsu², we used varied times of NaOH treatment and Giemsa staining. This produces a spectrum of banding patterns ranging from slides with chiefly G banded metaphases to slides with chiefly G/C band hybrids and then to slides with chiefly C banded metaphases, the last being produced with the longest treatment in NaOH (Fig. 1). It is evident that the G bands are not quite as resistant to NaOH denaturation as the C bands. The paracentric C bands in human chromosomes 1 and 16 also stain in the G band technique, however. Further, the enormous blocks of constitutive heterochromatin on the sex chromosomes of the field vole (*Microtus agrestis*) do not stain as C bands in ignition spread preparation, but they do stain heavily in the G band techniques³. Thus, the C band technique alone does not define constitutive heterochromatin clearly.

Identical human G banding patterns have been produced with proteolytic enzyme preparations of different specificity⁴⁻⁷. Differences in the nature of the protein are therefore not critical and access to the substrate may be more relevant. Wilhelm *et al.*⁸ examined the histone and nonhistone proteins of both extra-nucleolar chromatin and nucleolar-associated chromatin (which is rich in heterochromatin^{9,10}), and found that the striking structural differences between these two chromatin types were not reflected in their protein constitution. There is some evidence, however, to suggest that heterochromatin-associated protein may have a low degree of phosphorylation^{11,12}.

Protein in densely packed heterochromatin would be more inaccessible to enzyme attack than that in euchromatic chromosomal regions. It is probable that the more repetitive the DNA, the denser the packaging of the nucleoprotein. Prolonged proteolysis completely removes the G bands and the chromosomes become swollen, non-staining structures which still contain abundant DNA, as shown by autoradiography, suggesting that Giemsa is a protein stain. Many authors¹³⁻¹⁷ have ascribed Giemsa banding to the denaturation and differential renaturation of DNA sequences. It has been stated¹⁴ that the darkly staining G banded regions represent a DNA component of the chromosome, but there is no evidence that this DNA is itself Giemsa stained. In addition, although denaturation and renaturation of DNA occur in the banding methods using strong alkali treatment, these do not occur in those G banding methods using a neutral pH saline buffer or trypsin¹⁸.

Synthetic DNAs rich in A-T base pairs strongly enhance the fluorescence of quinacrine and a corresponding quenching is seen with G-C rich molecules^{19,20}. Proflavine, an acridine related to quinacrine, gives similar banding results²¹. In addition, the binding process of proflavine to DNA is similar, whether the chromatin is protein extracted with NaCl or not²². This suggests that quinacrine fluorescence in chromosomes is due to the DNA composition and is unaffected by chromosomal protein.

Comings²³ described families of sub-components of DNA in the Chinese hamster that tend to replicate at the same time. He also described a return to A-T richness as heterochromatin is replicated late in the S period. We believe that families of related sequences in fractions such as the "homogeneous main band DNA"²⁴ and the "intermediate reassociating repetitive DNA"²⁵ are prime candidates for localization in the intercalary constitutive heterochromatin of man. Human homogeneous main band DNA is A-T rich²⁶. There is also some evidence in man that the intensely quinacrine-fluorescing bands are also late replicating^{27,28}.

Comings²⁹ postulated a mechanism (cytosine methylation and later deamination) whereby heterochromatic regions may evolve into A-T rich sites. In the light of the above discussion, this mechanism would explain the similarities of the G and Q banding patterns. We propose that G bands are found at sites enriched with types of constitutive heterochromatin correlating with Q bands only in so much as this heterochromatin is A-T rich. The dissimilarities between the Q and G banding patterns are found at sites enriched with centromeric constitutive heterochromatin. These could be explained by the presence of highly repetitive DNA which has not become A-T rich because of a high proportion of non-methylatable G-C base pairs in the original sequence which was amplified into the repetitive DNA.

Our hypothesis of extraction of protein at differential rates seems to relate the results of the different banding techniques in man. Table 1 shows the differing resistance to alkali disruption of various constitutively heterochromatic regions.

Table 1 Differential Resistance of Constitutively Heterochromatic Regions to Alkali Disruption

Technique	Time of alkali treatment		C band method ²	Giemsa 11 ¹⁷
	ASG ¹⁴ trypsin ⁵	Alkali/saline ¹³		
Regions				
Increasing resistance to treatment	G bands	+	+	
	16 qh	+	+	
	1 qh	+	+	±
	Distal Yq		+	±
	9 qh		+	+
↓	Proximal Yp		±	+

+, Bands present; ±, bands sometimes present.

The reverse banding technique³⁰ has its probable mechanism in the selective disruption of A-T rich sites at the specific temperature (87° C) of the prestaining treatment. Protein in these disrupted areas would be more easily extracted than that at the more heat stable regions, which are relatively rich in G-C base pairs. Again, a prolonged treatment produces non-staining structures. A DNA sequence containing even 50% G-C base pairs would be relatively G-C rich, as compared with the A-T rich heterochromatin. It is quite probable that the darkly staining R bands contain largely euchromatic DNA.

A. DANIEL

P. R. L. C. LAM-PO-TANG

*Cytogenetics and Cell Biology Unit,
The Prince of Wales Hospital,
Randwich,
New South Wales 2031*

Received April 10; revised May 21, 1973.

- ¹ Comings, D. E., *Expl. Cell Res.*, **67**, 441 (1971).
- ² Arrighi, F. E., and Hsu, T. C., *Cytogenetics*, **10**, 81 (1971).
- ³ Cooper, J. E. K., and Hsu, T. C., *Cytogenetics*, **11**, 295 (1972).
- ⁴ Dutrillaux, B., De Grouchy, J., Finaz, C., and Lejeune, J., *C. r. hebdom. Séanc. Acad. Sci. Paris*, **273**, 587 (1971).
- ⁵ Seabright, M., *Lancet*, **ii**, 971 (1971).
- ⁶ Finaz, C., and De Grouchy, J., *Ann. Genet.*, **14**, 309 (1971).
- ⁷ Prieur, M., Dutrillaux, B., Rethore, M. O., and Lejeune, J., *Ann. Genet.*, **14**, 305 (1971).
- ⁸ Wilhelm, J. A., Ansevin, A. T., Johnson, A. W., and Hnilica, L. C., *Biochim. biophys. Acta*, **272**, 220 (1972).
- ⁹ Nagl, W., *J. cell. Sci.*, **6**, 87 (1970).
- ¹⁰ Mattoccia, E., and Comings, D. E., *Nature new Biol.*, **229**, 175 (1971).
- ¹¹ Dastugue, B., Tichonicky, L., and Kruh, J., *Biochimie*, **1435** (1972).
- ¹² Rickwood, D., Riches, P. G., and Macgillivray, A. J., *Biochim. biophys. Acta*, **299**, 162 (1973).
- ¹³ Drets, M. E., and Shaw, M. W., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2073 (1971).
- ¹⁴ Sumner, A. T., Evans, H. J., and Buckland, R. A., *Nature new Biol.*, **232**, 31 (1971).
- ¹⁵ Yunis, J. J., Roldan, L., Yasmineh, W. G., and Lee, J. C., *Nature*, **231**, 532 (1971).
- ¹⁶ Schnedle, W., *Chromosoma (Berl.)*, **34**, 448 (1971).
- ¹⁷ Bobrow, M., Madan, K., and Pearson, P. L., *Nature new Biol.*, **238**, 122 (1972).
- ¹⁸ de la Chapelle, A., Schröder, J., and Selander, R.-K., *Chromosoma (Berl.)*, **40**, 347 (1973).
- ¹⁹ Pachman, V., and Rigler, R., *Expl. Cell Res.*, **72**, 603 (1972).
- ²⁰ Weisblum, B., and De Haseh, P. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 629 (1972).
- ²¹ Caspersson, T., Zech, L., Wagh, U., Modest, E. J., and Simonsen, E., *Expl. Cell Res.*, **58**, 141 (1969).
- ²² Lawrence, J., and Louis, M., *Biochim. biophys. Acta*, **272**, 231 (1972).
- ²³ Comings, D. E., *Expl. Cell Res.*, **71**, 106 (1972).
- ²⁴ Corneo, G., Ginelli, E., and Polli, E., *J. molec. Biol.*, **48**, 319 (1970).
- ²⁵ Arrighi, F. E., Saunders, P. P., Saunders, G. F., and Hsu, T. C., *Experientia*, **27**, 964 (1971).
- ²⁶ Chen, T. R., and Ruddle, F. H., *Chromosoma (Berl.)*, **34**, 51 (1971).
- ²⁷ Ganner, E., and Evans, H. J., *Chromosoma (Berl.)*, **35**, 326 (1971).
- ²⁸ Allenderdick, P. W., Miller, D. A., and Miller, O. J., *Nature new Biol.*, **236**, 76 (1972).
- ²⁹ Comings, D. E., *Expl. Cell Res.*, **74**, 383 (1972).
- ³⁰ Dutrillaux, B., and Lejeune, J., *C. r. hebdom. Séanc. Acad. Sci. Paris*, **272**, 2738 (1971).

Androgen Metabolism in Rat Interstitial Tissue and Seminiferous Tubules

ALTHOUGH it is generally agreed that the interstitial (Leydig) cell of the testis is the main source of testosterone, which is essential for normal spermatogenesis, it has been suggested¹ that the seminiferous tubules may also be involved in steroid production.

Evidence that the tubules are capable of metabolizing certain steroids has come from Christensen and Mason² and Hall *et al.*³ who have separated rat testicular tubules and interstitial tissue by dissection and demonstrated that both tissue fractions can convert pregnenolone and progesterone to androstenedione and testosterone *in vitro*. Although the interstitial tissue was far more active than the seminiferous tubules (on a weight to weight basis) because tubules constitute the major portion of testicular mass, Christensen and Mason² concluded that as much as 10% of the testicular androgens may be produced in the seminiferous tubules.

Hall *et al.*³ and Cooke *et al.*⁴ have failed to demonstrate cholesterol metabolism in rat seminiferous tubules but side chain cleavage of cholesterol by the tubules has recently been demonstrated by Bass *et al.*⁵. It seems that the seminiferous tubules are of minimal importance in the conversion of cholesterol to pregnenolone, but it is possible that pregnenolone enters the tubules⁶ and is further metabolized there.

In order to determine the role of the seminiferous tubules in the later stages of testosterone biosynthesis, we have studied the metabolism of dehydroepiandrosterone (DHA), an inter-

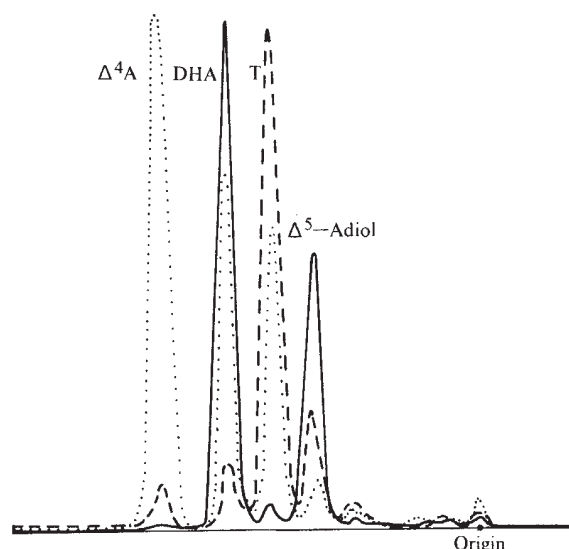


Fig. 1 Radiochromatogram scan of the metabolites formed from DHA-4-¹⁴C *in vitro* by various fractions of the adult rat testis. Thin layer chromatography was carried out on silica gel in dichloromethane : acetone, 6 : 1. The plate was developed twice. Δ^4 A, Androstenedione; DHA, dehydroepiandrosterone; Δ^5 -Adiol, androst-5-ene-3 β ,17 β -diol; T, testosterone. —, Seminiferous tubules; . . . , interstitial tissue; --- : whole testis.

mediate on the 5-ene pathway of testosterone production, in isolated interstitial tissue and seminiferous tubules, as well as in the intact testis, from the adult rat. Our results reaffirm the importance of the interstitial cell in androgen biosynthesis, but also reveal an important cooperative role of the tubules in determining the production of testosterone relative to other steroids.

Tubules and interstitial tissue from one testis of a 6-month-old Sprague-Dawley rat (weight 640 g) were separated in medium 199 buffered with HEPES, following the method of Hall *et al.*³. The tubules were washed six times with fresh medium to remove any free cells, and were examined under the dissecting microscope to check that there was no gross interstitial cell contamination. The interstitial tissue fraction contained short portions of tubules and free tubular cells. Dissection of one testis by this method took 2 h. The tunica was removed from the second testis, and the tissue kept in the same medium without dissection for 2 h. The cell viability, checked with Leishman green stain, was found to be greater than 70% in the interstitial tissue, much of the dead material consisting of damaged tubules rather than interstitial cells. Seminiferous tubules showed some uptake of the dye by peritubular cells where the tubules had been broken or damaged on dissection, but the cells extruding from the broken ends of tubules remained viable. The undissected testis did not take up the dye. Portions of each tissue fraction were processed for light microscopy when no contamination of the tubule fraction with interstitial cells could be seen.

Each tissue fraction (tubules, interstitial tissue, and intact testicular tissue) was divided into two equal portions for incubation. Thus, six flasks were set up, each containing tissue from half a testis. After removal of the tunica, half a testis weighed 850 mg, the corresponding tubule fraction 740 mg, and the interstitial tissue fraction (with some tubular contamination) 90 mg.

The contents of each flask were incubated for 2 h at 37° C with DHA-4-¹⁴C (1 μ Ci, 16 nmol) in 8 ml medium 199 buffered with HEPES at pH 7.4, containing 15% foetal calf serum. The reactions were stopped by adding dichloromethane. The metabolites were investigated by the conventional techniques of thin layer chromatography, derivative formation and recrystallization to constant specific activity.

The distribution of radioactivity after initial chromato-

graphy of lipid extracts from the incubations is shown in Fig. 1. The four principal peaks of activity correspond to DHA (unreacted substrate), androst-5-ene-3 β ,17 β -diol (androstenediol), androstenedione and testosterone (Fig. 2). The identities of these peaks were confirmed by recrystallization, and the percentage conversion of substrate to products, in each incubation, is shown in Table 1.

Table 1 Metabolism of DHA-4-¹⁴C *in vitro* by Seminiferous Tubules, Interstitial Tissue and the Intact Testis from Adult Sprague-Dawley Rat

Tissue type	DHA metabolized (%)					
	ST	ST	INT	INT	W	W
Total metabolism	39.3	49.1	68.9	78.4	86.8	67.8
Androstenediol	36.7	45.9	5.8	5.1	11.5	11.6
Androstenedione	0.8	0.9	30.4	34.2	12.4	15.2
Testosterone	1.2	2.1	25.2	32.5	55.4	35.4
Unidentified metabolites	0.6	0.2	7.5	6.6	7.5	5.6

Each incubation contained the relevant tissue from half a testis. ST: Seminiferous tubules; INT: interstitial tissue; W: intact testicular tissue, after removal of the tunica.

The principal metabolite of DHA in adult rat seminiferous tubules is androstenediol; the production of androstenedione and testosterone is very low. In contrast, in interstitial tissue, androstenedione and testosterone are the major metabolites, the production of androstenedione slightly exceeding that of testosterone. In undissected testicular tissue androstenedione and testosterone are again the major metabolites, but the production of testosterone greatly exceeds that of androstenedione. In addition, the production of androstenediol is greater than that found in interstitial tissue alone.

These results demonstrate that the seminiferous tubules contain a high level of 17 β -hydroxysteroid dehydrogenase, but a very low level of C₁₉ Δ^5 -3 β -hydroxysteroid dehydrogenase/isomerase activity. The production of testosterone and androstenedione in the isolated tubules is only 4% of that in the corresponding interstitial tissue under the same conditions. This low value cannot be accounted for by loss of tubules during dissection, or by damage to the tissue. The cell type responsible for Δ^5 -3 β -hydroxysteroid dehydrogenase isomerase activity within the tubules is unknown; it has been suggested that the Sertoli cell is involved^{1,2}. Although the tubules appeared free of interstitial cell contamination when examined microscopically, the possibility of interstitial cell contamination cannot be completely ruled out.

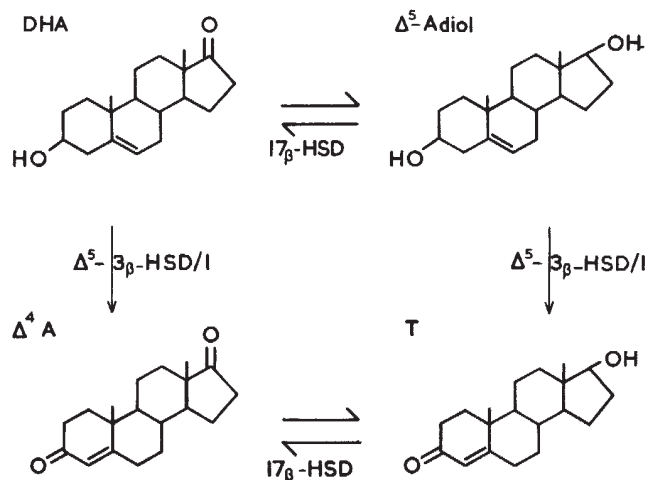


Fig. 2 The principal metabolites of DHA in the adult rat testis *in vitro*. DHA, Dehydroepiandrosterone; Δ^5 -Adiol, androst-5-ene-3 β ,17 β -diol; Δ^4 A, androstenedione; T, testosterone; 17 β -HSD, 17 β -hydroxysteroid dehydrogenase; Δ^5 -3 β -HSD/I, Δ^5 -3 β -hydroxysteroid dehydrogenase isomerase.

Incubations with isolated tubules and undissected testis yielded testosterone to androstenedione ratios between 2 and 5. This is comparable to the ratios observed in spermatogenic blood of the adult rat⁷. The increased production of testosterone from DHA by undissected testis, compared with the isolated interstitial tissue, may be due to the high 17 β -hydroxysteroid dehydrogenase activity within the seminiferous tubules.

These results confirm that the interstitial tissue of the testis is the principal site of conversion of 5-ene to 4-ene steroids. *In vitro* the seminiferous tubules have an important role in determining the relative production of androstenedione and testosterone. Whether the tubules have a similar role *in vivo* remains to be investigated.

GWEN RICHARDS
A. MUNRO NEVILLE

Department of Pathology,
Institute of Cancer Research,
Chester Beatty Research Institute,
Fulham Road,
London SW3 6JB

Received April 5, 1973.

- ¹ Lacy, D., and Pettitt, J., *Brit. med. Bull.*, **26**, 87 (1970).
- ² Christensen, A. K., and Mason, N. R., *Endocrinology*, **76**, 646 (1965).
- ³ Hall, P. F., Irby, D. C., and de Kretser, D. M., *Endocrinology*, **84**, 488 (1969).
- ⁴ Cooke, B. A., de Jong, F. H., van der Molen, H. J., and Rommerts, F. F. G., *Nature new Biol.*, **237**, 255 (1972).
- ⁵ Bass, J. J., Bell, J. B. G., and Lacy, D., *J. Endocr.*, **56**, 321 (1973).
- ⁶ Parvinen, M., and Niemi, M., *Steroidologia*, **2**, 129 (1971).
- ⁷ Bardin, C. W., and Peterson, R. E., *Endocrinology*, **80**, 38 (1967).

Methods of Examining Fibrinolysis in Plasma

IMMUNOLOGICAL methods have been used to estimate the levels of fibrinogen and fibrin degradation products in serum samples during thrombolytic therapy and in various clinical situations. A recent report¹ has suggested that methods such as the tanned red blood cell haemagglutination inhibition immunoassay² and latex aggregation³ measure mainly the products of fibrin degradation. We wish to report on techniques which have proved useful in differentiating between the degradation products of fibrinogen and fibrin clots in whole plasma systems *in vitro* and which have helped in confirming results obtained^{4,5} using purified fibrinogen and fibrin clots.

Human fibrinogen (Grade 4 from KABI AB, Stockholm) was used throughout these experiments and the human plasma used was freshly drawn into citrate from one of us (P. J. G.). Sodium dodecyl sulphate (SDS) acrylamide gel electrophoresis⁶ was performed on reduced and unreduced protein solutions. The acrylamide gels were autoradiographed using a 15 h contact time with Osray-M X-ray film. To check the nature of the fragments released from clotted fibrin and plasma fibrinogen during enhanced fibrinolysis in plasma, 5 μ Ci of ¹²⁵I-labelled fibrinogen⁷ was added to 1 ml of plasma and clotted with 2 NIH units of bovine thrombin (Diagnostic Reagents, Thame, Bucks.) for 30 min at 37° C. The radioactive plasma clot was wound on a wooden stick, washed with saline four times and placed in 1 ml of plasma. A second type of clotted fibrin was made by clotting 1 ml of a 0.3% fibrinogen solution (KABI Grade L) and 5 μ Ci of ¹²⁵I-labelled fibrinogen. This type of fibrin was also placed in 1 ml of plasma. One ml aliquots of ¹²⁵I-fibrinogen plasma were prepared with and without the two types of fibrin clots (non-radioactive). Sixty IU streptokinase (SK) in 20 μ l of 0.15 M NaCl were added to all systems and incubated for 6 h at 37° C. A separate ¹²⁵I-fibrinogen plasma sample was treated with urokinase (UK) for 6 h at 37° C. Aliquot samples from each test system were electrophoresed on 5% SDS acrylamide gels (Fig. 1).

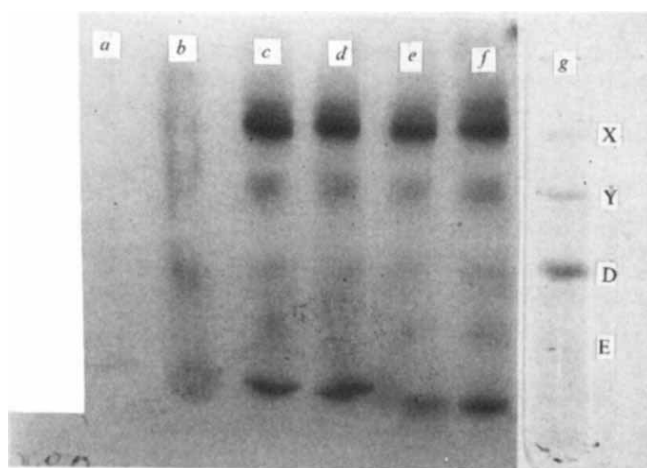


Fig. 1 Autoradiographs of SDS acrylamide gels (5%) showing the labelled fragments released from, a, ¹²⁵I-plasma clots in plasma by SK; b, ¹²⁵I-fibrin clots in plasma by SK; c, ¹²⁵I-plasma fibrinogen by SK in plasma containing plasma clots; d, ¹²⁵I-plasma fibrinogen by SK in plasma containing fibrin clots; e, ¹²⁵I-plasma fibrinogen by SK in plasma; and f, ¹²⁵I-plasma fibrinogen by UK in plasma; g, control group of reference fibrinogen fragments X, Y, D and E.

A control gel (Fig. 1g) shows the well characterized⁸⁻¹¹ groups of fibrinogen degradation products X, Y, D and E to which we wish to relate the fragments produced during degradation of fibrin clots and fibrinogen. During enhanced plasma fibrinolysis no radioactive material is released from the plasma clot while a clot made from purified fibrinogen releases low levels of a variety of compounds (Fig. 1a, b). Plasma fibrinogen in the presence of plasma clots and fibrin clots degrades mainly to a fragment similar in molecular weight to fragment X with lesser amounts of other components similar in size to Y, D and E (Fig. 1c, d). The concentration and range of degradation components are similar when ¹²⁵I-plasma fibrinogen is treated with SK and UK activated plasmin in the absence of clotted fibrin (Fig. 1e and f). Plasma fibrinogen, therefore, degrades mainly to fragment X and this reaction is not affected by the presence of fibrin clots.

It has been reported¹² that fibrin chain crosslinking catalysed by the plasma enzyme, Factor XIII, affects the susceptibility of fibrin to lysis with (UK). To assess why the plasma clot was less susceptible to lysis than the purified fibrin clot in plasma, both types of fibrin were examined for polypeptide chain composition before and after treatment with SK. The fibrins were washed in isotonic saline (0.2 M ϵ -aminocaproic acid (ϵ -ACA)) and treated overnight with 4 M urea (1% SDS 0.2 M β -mercaptoethanol) before electrophoresis in 7% SDS acrylamide gels (Fig. 2). A control mixture of the fibrin chains (α , β and γ , Fig. 2e) was used to relate the subunits in the test samples. It can be seen that the plasma clot (Fig. 2a) has all its γ chains crosslinked as γ - γ dimers and α chains crosslinked to α polymers (α^2). Albumin chain impurities can be detected in the α chain region of the gels even after exhaustive washing of plasma clots¹⁴. The clot prepared from purified fibrinogen has some γ - γ dimers and no α polymers. The chain composition of the plasma clot after its attempted lysis with plasmin (Fig. 2c) is the same as that before lysis (Fig. 2a) while the partly crosslinked fibrin clot residue (Fig. 2d) is made up of β and some γ intact chains, γ - γ dimers and fragments of the α chain (α' , molecular weight, 25,000). These have been described elsewhere¹⁵ as the initial α -chain residues which remain an integral part of non-solubilized fibrin.

Antiplasmins adsorbed into the totally crosslinked plasma clot might explain its resistance to lysis but thorough washing of these clots in saline reduces this possibility. We conclude that the presence of α -chain polymers, and to a lesser extent γ - γ chains, endowed these plasma clots with resistance to lysis (Fig. 1a) supporting recent subunit evidence⁴ which rational-

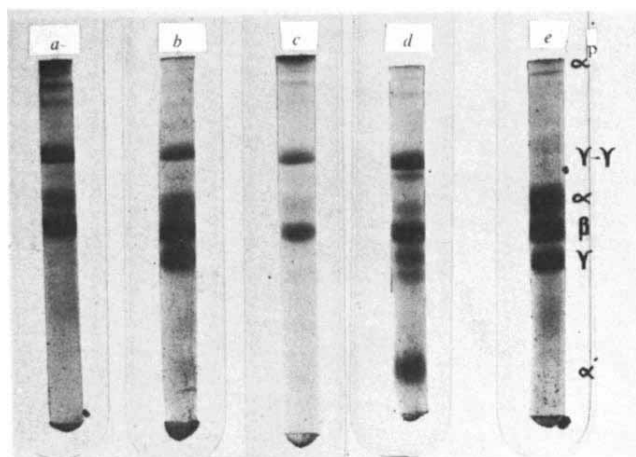


Fig. 2 SDS acrylamide gel (7.5%) electrophoretic patterns of the polypeptide chains of, *a*, totally crosslinked (TXL) plasma fibrin clots; *b*, partly crosslinked (PXL) purified fibrin clots; *c*, TXL plasma fibrin clots after SK activated fibrinolysis in plasma; *d*, PXL purified fibrin clots after activated fibrinolysis in plasma; *e*, reference chains of fibrin α , β and γ whose molecular weights are 65,000, 56,000 and 47,000 respectively¹⁵. The plasma fibrin clots, (*a*) and (*c*), contain albumin impurity in the α chain location¹⁴. α -Chain polymers which cannot enter the gel (α P); γ dimers (γ - γ); a fragment of the α chain of molecular weight about 25,000 (α'). Electrophoresis was for 15 h at 2 mA per gel, the gels were stained for 4 h in 1% Amido-black and destained overnight with large volumes of 7% acetic acid.

izes the observation¹⁶ that plasma clots produced in the presence of Ca^{2+} (necessary for Factor XIII activity and activation) are highly resistant to lysis by plasmin.

SDS acrylamide electrophoresis, autoradiography and immunoabsorption were used to examine the end products of the plasmin degradation of fibrinogen and fibrin in plasma. A ¹²⁵I-labelled plasma clot was treated in plasma with 60 IU SK and then five caseinolytic units of human plasminogen added, and incubated at 37°C for 4 h. An unlabelled clot was incubated in ¹²⁵I-fibrinogen plasma and treated similarly. The clots lysed rapidly and completely because of added plasminogen compared to the clots with no plasminogen added described above (Fig. 1). This supports the recent important role given¹⁷ to plasminogen perfusion in clot lysis. Aliquots of the lysates were absorbed with rabbit anti-human fibrinogen serum and the

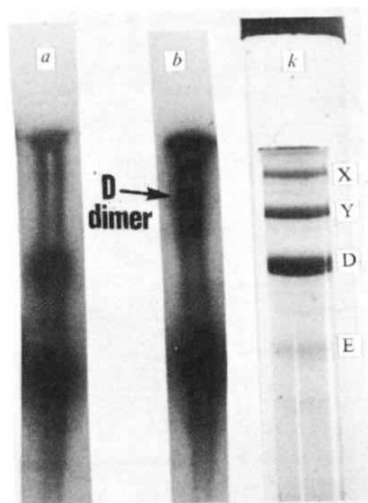


Fig. 3 Autoradiographs of SDS acrylamide electrophoresis gels (5%) showing the principal ¹²⁵I-labelled fragments absorbed by rabbit antihuman fibrinogen serum from plasminogen enriched SK treated: *a*, ¹²⁵I-plasma fibrinogen with non-labelled plasma clot; *b*, ¹²⁵I-plasma fibrin clot in non-labelled plasma. Pattern *k* shows the mobility of the known fibrinogen fragments X, Y, D and E for comparison.

washed immunoprecipitates were dissolved in 4 M urea (1% SDS), subjected to electrophoresis on SDS acrylamide gels and autoradiographed overnight (Fig. 3).

The labelled fragments released from plasma fibrinogen (Fig. 3*a*) had the same mobility as fragments D and E in the control marker mixture (Fig. 3*k*) while those released from labelled clots (Fig. 3*b*) can be described as a dimerized form of fragment D and fragment E. These dimerized forms of D have been shown⁴ to contain the γ - γ chain crosslinks of the originating fibrin clots. It seems that clotted fibrin does not release fragment D.

It is encouraging to realize that, in plasma, the use of SDS acrylamide gels coupled with the sensitive technique of autoradiography has helped us to confirm fibrinolysis findings^{4,5} obtained in purified systems. Methodology to specifically measure levels of D dimer in plasma would be useful to monitor thrombolytic therapy and/or observe the clearance of *in vivo* fibrin deposits. We suggest that immunoabsorption of fragment D dimer from plasma may be useful as a concentration step as the dimer would be present at low levels in a blood volume of 5 l during the lysis of normal-sized thrombi.

We thank Dr A. S. McFarlane of the Chemical Research Centre, Harrow, Middlesex, who supplied the ¹²⁵I-labelled fibrinogen and Mochida Pharmaceuticals, Tokyo, for the gift of urokinase.

P. J. GAFFNEY
M. BRASHER

National Institute for Medical Research,
Holly Hill,
London NW3 6RB

Received November 3, 1972; revised April 27, 1973.

- ¹ Gallimore, M. J., Tyler, H. M., and Shaw, J. T. B., *J. Clin. Path.*, **25**, 185 (1972).
- ² Merskey, C., Lazezari, P., and Johnson, A. J., *Proc. Soc. Exp. Biol. Med.*, **131**, 871 (1969).
- ³ Ferreira, H. C., and Murat, L. G., *Br. J. Haematol.*, **9**, 299 (1963).
- ⁴ Gaffney, P. J., and Brasher, M., *Biochim. Biophys. Acta*, **295**, 308 (1973).
- ⁵ Gaffney, P. J., *Fibrinogen and its Derivatives Symp.*, Warsaw, 1972. *Thrombosis Research* (in the press).
- ⁶ Weber, K., and Osborn, M., *J. Biol. Chem.*, **244**, 4406 (1969).
- ⁷ McFarlane, A. S., *J. Clin. Invest.*, **42**, 346 (1963).
- ⁸ Nussenzweig, V., Seligman, M., Pelmont, J., and Graber, P., *Ann. Inst. Pasteur*, **100**, 377 (1961).
- ⁹ Jamieson, G. A., and Gaffney, P. J., *Biochim. Biophys. Acta*, **154**, 96 (1968).
- ¹⁰ Marder, V. J., Shulman, N. R., and Carroll, W. R., *J. Biol. Chem.*, **244**, 2111 (1969).
- ¹¹ Budzynski, A. Z., Stahl, M., Kopec, M., Latallo, Z. S., Wegrzynowicz, Z., and Kowalski, E., *Biochim. Biophys. Acta*, **147**, 313 (1967).
- ¹² Gaffney, P. J., and Dobos, P., *FEBS Lett.*, **15**, 13 (1971).
- ¹³ McDonagh, R. P., jun., McDonagh, J., and Duckert, F., *Br. J. Haematol.*, **21**, 323 (1971).
- ¹⁴ Finlayson, J. S., and Morton, R. O., *Clin. Chim. Acta*, **36**, 254 (1972).
- ¹⁵ Gaffney, P. J., *Nature new Biol.*, **234**, 281 (1971).
- ¹⁶ Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, **58**, 485 (1933).
- ¹⁷ Chesterman, C., Allington, M., and Sharp, A., *Nature new Biol.*, **238**, 15 (1972).

Unique Amyloid Protein Subunit common to Different Types of Amyloid Fibril

It has been shown by Glenner *et al.*¹ that certain amyloid fibrils, chiefly derived from primary amyloidosis or amyloidosis associated with myelomatosis, consist of immunoglobulin chains or their fragments. The principal component of these fibrils seems to be derived from the variable part of monoclonal κ or λ light chains (Bence-Jones proteins). In addition, a "non-immunoglobulin" amyloid fibril protein with a molecular weight of 85,000, named by us protein AS (amyloid

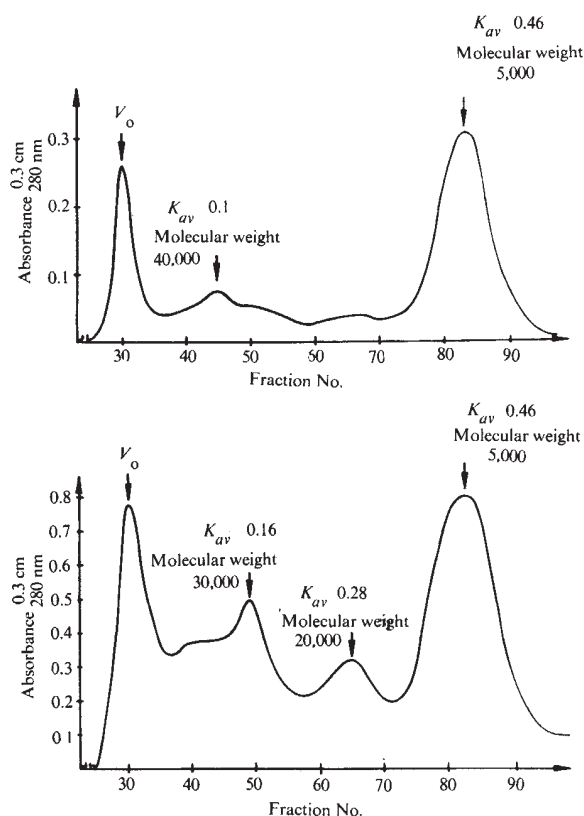


Fig. 1 Gel filtration of amyloid XVIII and amyloid XIII on a 3.2×96.5 cm 'Sephadex' G100 column with $V_0 = 157$ ml. Elution rate: 8.1 ml h^{-1} . Elution buffer: 5 M guanidine-1 N acetic acid; temperature: 23°C . Fraction volume 5.4 ml. Samples: amyloid XVIII: 10 ml, absorbance $\frac{1 \text{ cm}}{280 \text{ nm}} = 30$; amyloid XIII: 15 ml, absorbance $\frac{1 \text{ cm}}{280 \text{ nm}} = 30$.

subunit), which comprises an essential part of some secondary amyloid fibrils, has been detected by Benditt *et al.*² and by other groups³⁻⁵. The protein AS is found in secondary amyloid, chiefly associated with chronic inflammations, and has been suggested to be a fragment of a hitherto unknown larger protein⁴. Except for the detection of some light chain material connected with amyloid fibrils of the "non-immunoglobulin" type^{6,7}, no chemical link between the primary ("light chain") and secondary ("non-immunoglobulin") classes of amyloid substances has been established. On the contrary, the question has been raised whether there are two completely different chemical classes of amyloid⁸.

We now report the first demonstration of protein AS in a patient with amyloidosis associated with Waldenström's macroglobulinaemia with typical serum IgM M-components and Bence-Jones proteinuria and in other cases classified as primary amyloidosis. Furthermore, a serum component antigenically related to the amyloid protein AS was detected both in patients with secondary amyloidosis and also in several cases of primary or myeloma/macroglobulinaemia associated amyloidosis. The findings indicate that the protein AS may be a unique common constituent of amyloid.

The study included two males, J. B., 67 yr, and J. N., 62 yr, who had typical Waldenström's macroglobulinaemia with progressing anaemia and lymph node enlargement and massive infiltration of lymphoid cells in bone marrow and lymphoid tissue. Monoclonal IgM in one patient (J. B.) 22 mg ml^{-1} of κ type, in the other (J. N.) 64 mg ml^{-1} of λ type, was found in serum, and both patients had significant Bence-Jones proteinuria with κ and λ light chains respectively. One patient (J. B.) developed generalized amyloidosis and died in uraemia. At autopsy, massive amyloidosis was found in bone marrow, lymphoid tissue and various parenchymal organs. The

liver (3,800 g) consisted of 80% amyloid, as estimated on the basis of Congo red positive material in tissue sections, and was chosen for preparation of amyloid fibrils (amyloid XVIII). From the other patient (J. N.) enlarged lymph nodes were removed from the axilla by surgery and used as source for amyloid fibrils (amyloid XIX). Amyloid from some other cases of primary amyloidosis and eight cases of secondary amyloidosis was also studied. All available sera from patients with amyloidosis and several from other disease groups were also studied.

Amyloid fibril suspensions were prepared according to Pras *et al.*⁹ and treated with guanidine or sodium hydroxide followed by gel filtration on 'Sephadex' G100 and G25 as previously described^{7,10}. Antiserum was obtained by immunizing rabbits with crude alkali-treated amyloid (DAM)⁷. Amino acid composition and partial N-terminal amino acid sequence analysis by Edman degradation was performed as described by Husby *et al.*¹⁰.

In all eight instances so far studied, degraded amyloid from secondary amyloidosis showed a consistent and typical elution pattern (Fig. 1) (amyloid XIII). This pattern was different in some of the primary amyloidosis cases studied, which in particular lacked the pronounced last peak, where the non-immunoglobulin protein AS was found in secondary amyloid. The gel filtration pattern of guanidine treated amyloid XVIII from the Waldenström's macroglobulinaemia case J. B. was, however, very similar to that of secondary amyloid XIII (Fig. 1). A distinct peak was eluted at $K_{av} 0.46$, which is exactly where the non-immunoglobulin protein AS was eluted in the cases of secondary amyloidosis.

Antigenic identity was also demonstrated when crude DAM or purified protein AS of amyloid I, derived from a patient with amyloidosis secondary to juvenile rheumatoid arthritis⁷ and amyloid XVIII, was tested in double immunodiffusion against anti-DAM I or anti-DAM XVIII. Crude DAM gave two precipitation lines, while protein AS showed one line fusing with that of the crude DAM appearing closer to the antiserum well (Fig. 2a).

The amino acid composition of material eluted with $K_{av} 0.46$ of amyloid XVIII (J. B., Table 1) was very similar to that of secondary amyloid I. Furthermore the five N-terminal amino acids were Arg-Ser-Phe-Phe-Ser, which are identical to the corresponding N-terminal amino acids of protein AS of secondary amyloid I. Thus, by gel filtration, immunodiffusion experiments, amino acid composition and N-terminal sequence analysis, amyloid XVIII from Waldenström's macroglobulinaemia was shown to contain significant amounts of protein AS, which was similar to that of secondary amyloidosis.

When DAM from the other Waldenström patient (J. N., amyloid XIX) and from two other cases (amyloid III and VII) of primary amyloidosis were tested antigenically, a precipitation line which fused with that of protein AS was obtained, demonstrating protein AS-like material in these preparations. In two cases (amyloid XIX and amyloid VII from a case of primary amyloidosis) no protein AS was eluted at $K_{av} 0.46$ from

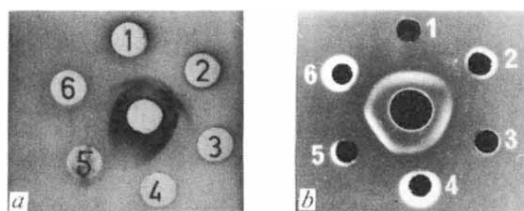


Fig. 2 a, Anti-DAM in central well tested against DAM I (1), DAM XVIII (2), protein AS XVIII (3), and protein AS I (6). The other wells (4 and 5) were filled with saline. Stained with amido black. b, Anti-protein AS in central well tested against DAM XVIII (1), serum from a case with secondary amyloidosis (2), DAM XIX (3), serum J. B. (4), protein AS I (5), and serum J. N. (6).

Table 1 Amino Acid Composition of Protein AS

	Residues per 100 residues	
	XVIII	I
Asp	13.3	13.8
Thr	0.74	0.44
Ser	6.96	7.06
Glu	8.55	8.42
Pro	2.02	1.64
Gly	11.3	11.9
Ala	16.4	15.9
Cys/2*	0.4	0.37
Val	1.73	1.45
Met	3.02	2.80
Ile	3.72	3.81
Leu	2.57	2.08
Tyr	5.69	5.50
Phe	7.96	8.86
His	2.03	2.59
Lys	3.41	2.91
Arg	10.2	10.9

Tryptophan was not determined.

* Cys/2 was determined as cysteic acid after performic acid oxidation.

guanidine treated material. Such material could be released and was eluted at K_{av} 0.46, however, after treatment of the amyloid with sodium hydroxide. Thus protein AS was demonstrated in several cases of so-called primary or myeloma/macroglobulinaemia associated amyloidosis.

With several good antisera available to detect the two principal components of amyloid, a search for amyloid-like material in human sera was attempted. One antiserum in particular (anti-DAM XVIII) reacting chiefly with protein AS gave a specific precipitation line with many human sera, particularly those of amyloidosis patients. These lines fused with that obtained with purified protein AS from amyloid fibril preparation (Fig. 2b). The precipitation reactions could also be blocked by adding purified protein AS to the antiserum. The positive sera came from various groups of patients (Table 2). These included sera of thirty-nine out of forty-one patients with amyloidosis amongst which were the two Waldenström's macroglobulinaemia cases, J. B. and J. N., in addition to patients with amyloidosis associated with myelomatosis and various conditions classified as either primary or secondary amyloidosis. A serum component related to protein AS has thus been found both in patients with primary and secondary amyloidosis. Several patients with diseases frequently complicated by amyloidosis were also positive. In contrast, isolated myeloma proteins, Bence-Jones proteins and normal sera were negative, except for a weak positive reaction in a single normal serum. It is, however, possible that protein

AS is present in very small amounts, not detectable by our methods, in normal sera.

Testing of positive sera after gel filtration ('Sephadex' G150) showed that the molecular weight of the protein AS related serum component is approximately 100,000, and immunoelectrophoresis of the sera against anti-DAM revealed an electrophoretic mobility in the α - β globulin region.

The amyloid fibril protein AS thus seems to be more widely distributed among different amyloid substances than thought originally and is not restricted to amyloidosis associated with inflammatory conditions. The classification of amyloid fibrils into an immunoglobulin (light chain) type related to primary or myeloma/macroglobulinaemia associated amyloidosis and a non-immunoglobulin type related to secondary amyloidosis is therefore not consistent in all cases. While protein AS was always found in secondary amyloidosis, this protein on several occasions also appeared in primary or myeloma/macroglobulinaemia associated amyloidosis, either directly in the amyloid substance or as a protein AS-related component in the serum. Thus protein AS may be a unique component common to different types of amyloidosis. The detection of protein AS in the serum of the vast majority of patients with amyloidosis may prove to be a valuable diagnostic tool in amyloidosis and possibly enable us to detect the precursor state of this serious disease.

G. HUSBY
J. B. NATVIG
T. E. MICHAELSEN

*Institute of Immunology and Rheumatology,
Rikshospitalet University Hospital*

K. SLETTEN

*Institute of Biochemistry,
University of Oslo*

H. HÖST

*The Norwegian Radium Hospital,
Oslo 1*

Received April 4; revised May 7, 1973.

- Glenner, G. G., Terry, W., Harrada, M., Isersky, C., and Page, C., *Science*, **172**, 1150 (1971).
- Benditt, E. P., Eriksen, N., Hermanson, N. A., and Ericsson, L. H., *FEBS Lett.*, **19**, 169 (1971).
- Ein, D., Kimura, S., and Glenner, G. G., *Biochem. biophys. Res. Commun.*, **46**, 498 (1972).
- Franklin, E. C., Pras, M., Levin, M., and Frangione, B., *FEBS Lett.*, **22**, 131 (1972).
- Husby, G., Sletten, K., Michaelsen, T. E., and Natvig, J. B., *Nature*, **238**, 187 (1972).
- Levin, M., Franklin, E. C., Frangione, B., and Pras, M., *J. clin. Invest.*, **51**, 2773 (1972).
- Husby, G., and Natvig, J. B., *Clin. exp. Immun.*, **10**, 635 (1972).
- Benditt, E. P., and Eriksen, N., *Am. J. Path.*, **65**, 231 (1971).
- Pras, M., Schubert, M., Zucker-Franklin, D., and Franklin, E. C., *J. clin. Invest.*, **47**, 924 (1968).
- Husby, G., Sletten, K., Michaelsen, T. E., and Natvig, J. B., *Scand. J. Immun.*, **1**, 393 (1972).

Table 2 Presence of Protein AS-related Serum Component

Diagnosis	No. positive/No. tested	
	Total	With known amyloidosis
Primary amyloidosis	7/7	7/7
Myelomatosis	47/88	3/4*
Macroglobulinaemia Waldenström	8/8	3/3
Rheumatoid arthritis	25/46	8/9*
Juvenile rheumatoid arthritis	20/38	8/8
Systemic lupus erythematosus	5/23	—
Cirrhosis of the liver	15/41	1/1
Ulcerative colitis	3/4	3/3
Familial Mediterranean fever	1/1	1/1
Cystic fibrosis of the pancreas	1/1	1/1
Medullary carcinoma of the thyroid	1/1	1/1
Other malignant diseases	3/3	3/3
Normal controls	1/70	—

* Patients with amyloidosis without protein AS-related serum component had severe liver and kidney conditions with hypoproteinaemia.

Trypanosome Identification by Electrophoresis of Soluble Enzymes

It is now generally accepted that interspecific differences are the result of inherited variations, developed during the course of evolution, in the structures (and amounts) of the proteins of the species concerned. Thus, although organisms of many diverse types possess cytochrome *c*, the cytochrome *c* molecules differ in structure, from organism to organism, to an extent which is related to the time that has elapsed since divergence from a common ancestor¹, and the same is true for the haemoglobins of animals². Intraspecific differences are the outward manifestations of less profound modifications affecting only a few of the proteins. Thus some human racial

groups differ in the incidence of certain of the variant forms of plasma proteins and red cell enzymes, and also in the incidence of some of the blood group types (which are now recognized as resulting from the activities of variant enzymes)³. Clearly, if analogous proteins in different strains of a species could be characterized in some way, such as by electrophoresis, which shows up small structural differences, biochemical classification of the different strains would be possible, and this could be of some value as a taxonomic tool. Some advances along these lines have already been made: in the field of protozoology for instance, different strains as well as different species of the malarial parasite were recently reported to differ, to a lesser and greater degree respectively, in electrophoretic characteristics⁴.

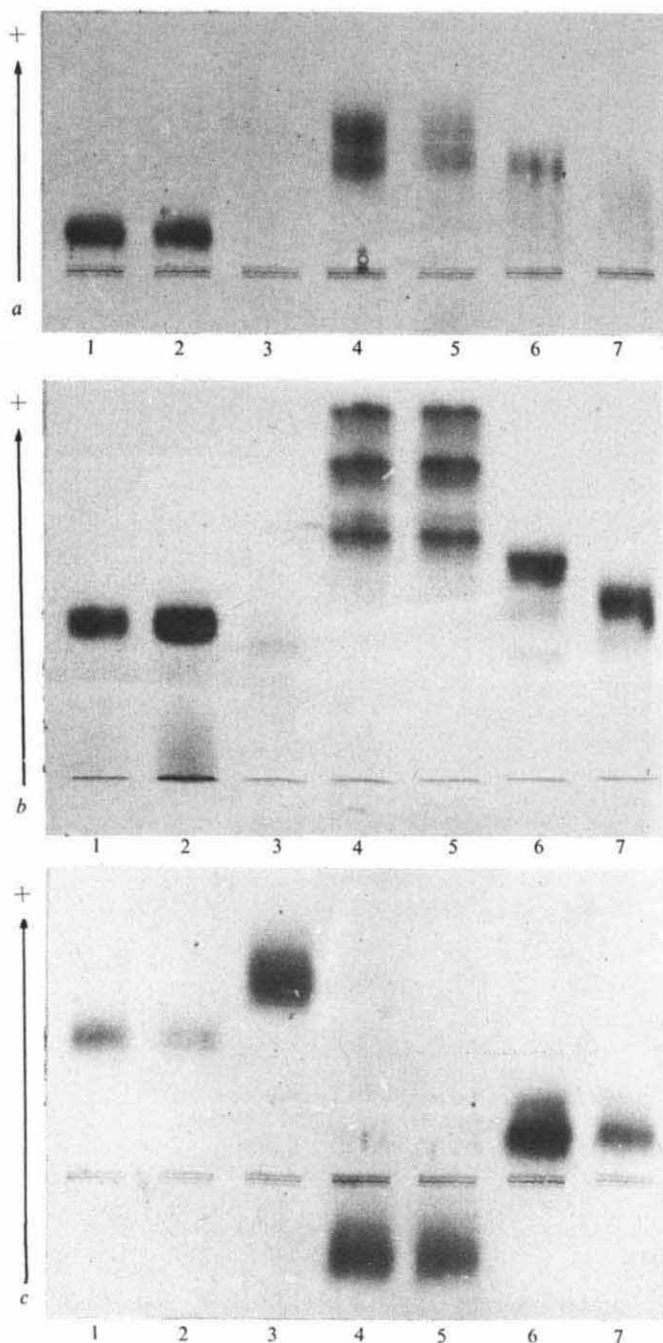


Fig. 1 Starch-gel electrophoretic patterns for seven different trypanosomal soluble extracts. A potential gradient of 15 V cm^{-1} of gel length was used, for a period of 4 h. *a*, Stained for G6PD; *b*, for ME; *c*, for PGI. Positions: 1, *T. lewisi* (Winches II); 2, *T. lewisi* (Molteno B₃); 3, *T. congolense* (1/148) (recently transmitted by tsetse fly); 4, *T. vivax* (Liverpool Swain strain); 5, *T. vivax* (Desowitz strain); 6, *T. b. brucei* (8/18) (recently transmitted by tsetse fly); 7, *T. b. brucei* (Ob₂).

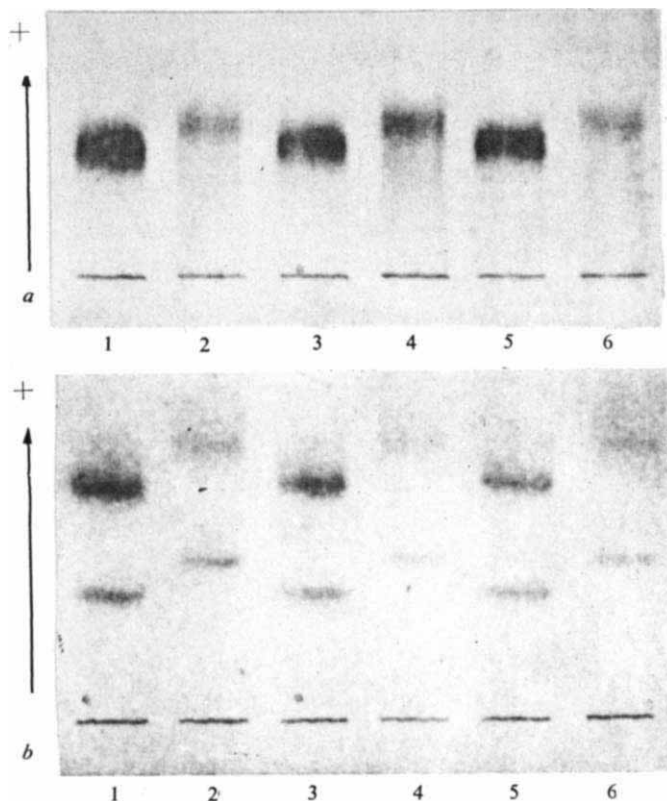


Fig. 2 Starch-gel electrophoretic patterns for soluble extracts from a *T. b. brucei* strain and a *T. b. rhodesiense* strain. A potential difference of 15 V cm^{-1} of gel length was used, for a time of 4 h. *a*, Stained for G6PD; *b*, for ME. Positions: 1, 3 and 5, *T. b. brucei* (Ob₂); 2, 4 and 6, *T. b. rhodesiense* (21).

We report here some well-defined differences in the electrophoretic behaviour of three soluble enzymes derived from four different trypanosome species, and some less well-defined differences between strains from within those species. This report, together with that of Kilgour⁵ on trypanosome aminotransferases, suggests that a systematic study of enzyme electrophoretic mobilities and relative activities will yield a new procedure for the identification of the different trypanosome species and possibly also for the identification of different strains.

Soluble extracts⁵ from some long term laboratory trypanosome strains were subjected to horizontal thin-layer starch-gel electrophoresis by a procedure essentially as described by Wraxall and Culliford⁶. The gels were then specifically stained for glucose-6-phosphate dehydrogenase (G6PD), the NADP-linked decarboxylating malic enzyme (ME), and phosphoglucose isomerase (PGI). G6PD was visualized by a procedure⁷ whereby the gel was incubated with glucose-6-phosphate, NADP, phenazine methosulphate (PMS) and the tetrazolium salt MTT: the NADPH_2 formed by the enzymatic reaction, in the presence of the hydrogen carrier PMS, reduced the MTT to a deep blue insoluble formazan which deposited at the sites of G6PD activity. ME was stained by a parallel procedure: the gel was incubated with a mixture containing malate, NADP, PMS and MTT: the blue formazan precipitated at the sites of ME activity. PGI was visualized by incubation with fructose-6-phosphate, added commercial G6PD, NADP, PMS and MTT: glucose-6-phosphate was first formed at the sites of PGI activity: it then reacted with the NADP in the presence of the added G6PD to form NADPH_2 which, using PMS, reduced the MTT to its deep blue insoluble derivative⁸.

From the results shown in Fig. 1 the four trypanosome species *Trypanosoma lewisi*, *T. congolense*, *T. vivax* and *T. brucei brucei* differ from each other quite clearly in the electrophoretic behaviour of the three enzymes reported.

Some differences in levels of enzymatic activity are also very apparent from Fig. 1: *T. congolense* is characterized by a relatively high PGI activity and an almost absent G6PD and ME. As would be expected, differences between strains of the same species were not generally so evident, but differences in the ME mobilities of two *T. b. brucei* strains are shown in Fig. 1b (positions 6 and 7). Small differences were observed in both the ME and the G6PD electrophoretic mobilities when a *T. b. brucei* strain and a *T. b. rhodesiense* strain were run alongside each other (Fig. 2a, b).

We thank Dr D. G. Godfrey of the Lister Institute for the trypanosome extracts and the Overseas Development Administration of the Foreign and Commonwealth Office for financial support.

I. A. BAGSTER
C. W. PARR

Department of Biochemistry,
The London Hospital Medical College,
London E1 2AD

Received March 16, 1973.

- ¹ Dickerson, R. E., *Sci. Amer.*, **226**, 58 (1972).
- ² Ingram, V. M., *Nature*, **189**, 704 (1961).
- ³ Giblett, E. R., in *Genetic Markers in Human Blood* (Blackwell, Oxford and Edinburgh, 1969).
- ⁴ Carter, R., and Voller, A., *Trans. R. Soc. trop. Med. Hyg.*, **67**, 14 (1973).
- ⁵ Kilgour, V., *Nature new Biol.*, **244**, 69 (1973).
- ⁶ Wraaxall, B. G. D., and Culliford, B. J., *J. foren. Sci. Soc.*, **8**, 81 (1968).
- ⁷ Fildes, R. A., and Parr, C. W., *Biochem. J.*, **87**, 45P (1963).
- ⁸ Carter, N. D., and Parr, C. W., *Nature*, **216**, 511 (1967).

Photomicrography of Corneal Endothelial Cells *in vivo*

We have described the principles of operation for a microscope designed to examine objects embedded in highly reflecting or scattering biological materials¹. We present here the first successful application of this device to the observation of endothelial cells lining the inside of the cornea.

The corneal endothelium is of crucial importance in maintaining the transparency of the cornea². Observation of corneal endothelial cells *in vivo*, using a slit-lamp microscope, began about 1920 (ref. 3), and is now a well established art. But useful magnification is typically limited to a maximum of about $\times 80$, though in practice magnifications are usually considerably below this maximum³. For research purposes in particular, it is desirable to observe and to photograph corneal endothelial cells at higher magnifications than can be obtained with standard slit-lamp microscopy. For this reason, Maurice⁴ developed a split-objective microscope for observing functioning endothelial cells at magnifications of around $\times 500$. For most convenient operation and focusing of Maurice's microscope at the highest magnifications, it is necessary to make physical contact between the epithelial surface of the cornea and the objective, either through a coverglass or a liquid medium. Maurice and his colleagues have photographed functioning corneal endothelial cells in enucleated, intact eyes^{4,5}. Using our microscope, we have been able to repeat and to extend these observations, observing individual corneal endothelial cells and some of their organelles at high magnifications in the eyes of living bullfrogs, for periods of more than 30 min. Observations were made without physical contact between the objective and the eye.

The optics of our laser microscope are so designed that at any instant, light reflected from only one point within the object is transmitted to a photomultiplier⁶. The amplified output of the photomultiplier intensity-modulates the electron beam of an oscilloscope. By scanning the laser beam with the objective lens,

and scanning the intensity-modulated electron beam in synchrony with the objective lens, a two-dimensional image is displayed on the oscilloscope of the reflectivities of points lying within an object at a given fixed depth.

The objective lens we have found most satisfactory is a Zeiss LD-Epiplan lens, $\times 40$, NA 0.60, with a working distance of 3.1 mm. We have used a continuous wave 5 mW He-Ne laser with a wavelength of 0.6328 μm . Only about a tenth of the laser output was necessary for observation of the endothelial cells. The levels of light at the cornea (and at the retina) during our examination of the corneal endothelium were well below threshold for damage to these tissues⁷⁻⁹.

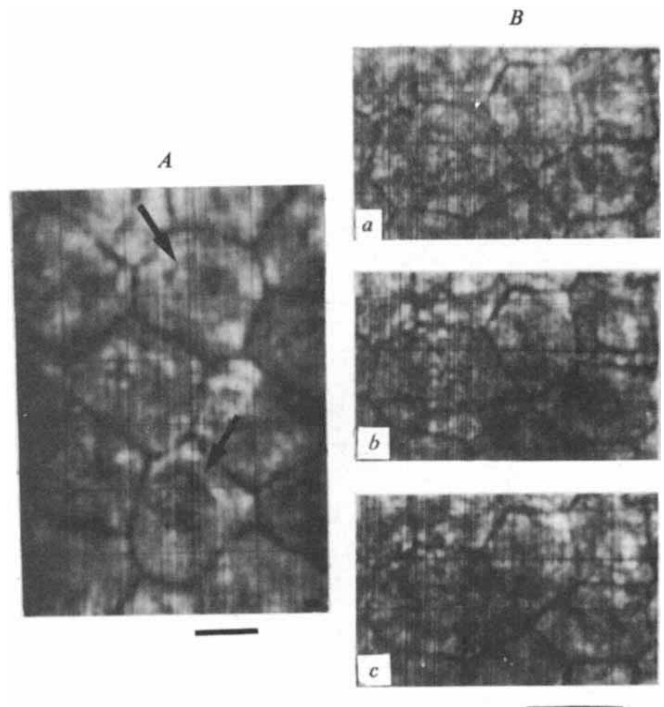


Fig. 1 *A*, Corneal endothelial cells in an unstained, unfixed intact eye of a living bullfrog. Arrows indicate outlines of the nuclei in two of the cells. Calibration mark, 25 μm . *B*, Three photographs of a group of corneal endothelial cells in an unstained, intact eye of a living bullfrog. *b*, Taken 15 min after *a*; *c*, taken 23 min after *a*. Calibration mark, 50 μm .

Figure 1*A*, which is a reproduction of a 'Polaroid' photograph of an oscillographic display, shows corneal endothelial cells within the eye of a living bullfrog, *Rana catesbeiana*. The bullfrog was immobilized by removing its brain and spinal cord. It was possible to observe a vigorous flow of blood in the vessels of the iris, both before and after this photograph was taken. Outlines corresponding to the nuclei of some of the endothelial cells are visible (arrows).

Figure 1*B* shows a series of three photographs of a group of corneal endothelial cells taken in another living bullfrog. The second picture (*b*) was taken 15 min after the first (*a*); the third (*c*), 23 min after the first. No gross differences in the arrangement or morphology of these cells or their nuclei occurred during this period.

We found, however, that in intact bullfrog eyes cooled to 4° C, sharp outlines of corneal endothelial cells, as seen in Fig. 1*A* and *B*, cannot be observed. Instead, only ghosts of outlines are seen, seemingly masked by highly reflecting regions at or near the plane of the endothelial cells. Whether these highly reflecting regions relate to changes in the morphology of the corneal endothelial cells themselves, or, perhaps, to changes in the hydration of Descemet's membrane immediately in front of the endothelial cell layer, is not known. These changes are,

however, reversible and, as the eye warms to room temperature, the usual configuration of the corneal endothelial cells can be seen after warming for about 30 min. These changes may be related to electrical and ionic events associated with decreased light transmission in cooled corneas¹⁰.

This work was supported by a National Science Foundation grant. M. D. E. holds a Research Scientist development award from the National Institutes for Mental Health. We thank Dr J. A. Zadunaisky for advice and encouragement.

PAUL DAVIDOVITS

Department of Engineering and Applied Science

M. DAVID EGGER

Department of Anatomy,
Yale University,
New Haven, Connecticut 06520

Received May 4, 1973.

¹ Davidovits, P., and Egger, M. D., *Nature*, **223**, 831 (1969).

² Stocker, F. W., *The Endothelium of the Cornea and its Clinical Implications* (C. C. Thomas, Springfield, Illinois, 1971).

³ Vogt, A., *Graefes Arch. Ophthalm.*, **101**, 123 (1920).

⁴ Maurice, D. M., *Experimentia*, **24**, 1094 (1968).

⁵ Hoefle, F. B., Maurice, D. M., and Sibley, R. C., *Arch. Ophthalm.*, **84**, 741 (1970).

⁶ Davidovits, P., and Egger, M. D., *Appl. Opt.*, **10**, 1615 (1971).

⁷ Sliney, D. H., in *Laser Applications in Medicine and Biology* (edit. by Wolbarsht, M. L.), 163 (Plenum Press, New York, 1971).

⁸ Michaelson, S. M., *Proc. I.E.E.E.*, **60**, 389 (1972).

⁹ Adams, D. O., Beatrice, E. S., and Bedell, R. B., *Science*, **N.Y.**, **177**, 58 (1972).

¹⁰ Zadunaisky, J. A., in *Electrophysiology of Epithelial Cells* (edit. by Giebisch, G.), 225 (F. K. Schattauer Verlag, New York, 1971).

Further Investigations into the Teratogenic Potential of Imperfect Potatoes

In a preliminary report¹ on the effects of feeding a dietary supplement of "blighted" potato concentrate to pregnant marmosets (*Callithrix jacchus*) reference was made to further experiments then in progress. The results of these supplementary experiments are now available.

The potatoes used in the experiments which form the substance of this report were all prepared and fed under identical conditions to those employed in the original "blighted" potato trial previously reported¹. The varieties used were normal domestic potato, potatoes rejected by graders in the food-processing industry (these were blighted, damaged and discoloured in the ratio 3:2:1) and tubers infected in the laboratory with *Erwinia carotovora*, a rotting organism known to produce rishitin and phytoalexin in the tubers. The results of this experiment are recorded in Table 1. While the offspring of both "domestic" and "reject" potato groups proceeded to term, those in the "Erwinia" group were obtained by hysterotomy to prevent the loss by parental destruction of any foetus born with gross anomalies. No gross anomalies were found in any

group, but behavioural defects comprising excessive clinging, abnormal play patterns, and inability to wean by 4 months were observed in three sets of twins born to mothers all fed with batch 1 of the "reject" potato concentrate. These abnormalities of behaviour have not previously been observed in colony-bred marmosets.

Terpenoid derivatives such as rishitin and phytoalexin, and steroid glycoalkaloids including solanine, have been implicated in a hypothesis concerning the occurrence of spina bifida and anencephaly in man². In view of this and the results of the previous animal experiment¹, all samples involved in the present and earlier trials were analysed for total glycoalkaloids by accepted methods^{3,4}, for terpenoids by gas chromatography⁵ and chloroform extracts were compared by thin layer chromatography (Table 2).

Table 2 Analysis of Potato Samples for Rishitin, Phytoalexin and Total Glycoalkaloid Content in $\mu\text{g g}^{-1}$ Freeze Dried Material

Sample	Rishitin	Phytoalexin	Total glycoalkaloids
Kerr's Pink "blighted"	Trace	None	20
<i>Erwinia</i> -infected, batch 1	89	69	1
<i>Erwinia</i> -infected, batch 2	56	88	2
Industry-reject, batch 1	8	Trace	38
Industry-reject, batch 2	5	1	9
Industry-reject, batch 3	None	None	3
Domestic controls, batch 1	Trace	None	10
Domestic controls, batch 2	None	Trace	14

Although the levels of glycoalkaloids in Kerr's Pink "blighted" potato concentrate were higher than in the controls, it is unlikely that this difference alone is significant in relation to the cranial defects observed in the earlier experiment as higher levels in the "reject" batch 1 mentioned did not produce the same effect. Rishitin and phytoalexin, though present at high levels in the "Erwinia" concentrate, produced no malformations comparable with the cranial osseous defects previously observed.

To check the possibility that organic mercury might be involved in the production of cranial defects⁶ analysis of the mercury content was carried out. The highest level, in Kerr's Pink potato concentrate, was 0.004 mg kg^{-1} fresh weight, and just within the normal range.

Shepard⁷ suggested that cytochalasin, which had been implicated in neural tube malformations in chick embryos⁸, may be present in blighted potato. Analysis of all samples for cytochalasin activity by Carter (personal communication) revealed no activity in any sample except for the original Kerr's Pink potato concentrate. A weak but typical effect (binucleation in cultured L cells⁹) was found, representing a total daily dose per marmoset in the original trial¹ equivalent in activity to $3 \mu\text{g}$ of cytochalasin B. The source of this activity could have been fungal pathogens such as *Phoma exigua* and *Helminthosporium solani*, known cytochalasin producers, found in mouldy seed potatoes¹⁰.

Table 1 Comparison of Offspring in Potato Feeding Trials

	"Domestic" potato Group 1	"Industry reject" potato Group 2	<i>Erwinia</i> potato Group 3
No. of treated females	6	7	5
Offspring this pregnancy	10 (4 × 2, 2 × 1)	12 (5 × 2, 2 × 1)	13 (3 × 3, 2 × 2)
Sexes this pregnancy	Seven males, three females	Six males, six females	Seven males, six females
Offspring previous pregnancy	11 (5 × 2, 1 × 1)	12 (5 × 2, 2 × 1)	11 (4 × 2, 1 × 3)
Sex previous pregnancy	Six males, five females	Six males, six females	Six females, five males
Gross anatomical defects	0	0	0
Behavioural irregularities	0	6 (3 × 2)	Not examined

The significance of the behavioural abnormalities cannot be assessed in full until the animals have been observed to develop to sexual maturity. The higher levels of glycoalkaloids in the "reject" batch 1 fed to these mothers may be of significance: solanine is toxic to man¹¹ but not apparently teratogenic in the rat¹².

The only safe conclusion that can be drawn from these supplementary experiments is that the cranial defects were not reproduced. Some correlations can be made between the chloroform extract analyses obtained by thin layer chromatography. Components, as yet unidentified, but common to both the original "blighted" potato and the present industry reject samples, were found; the significance of this remains to be established. Parameters for assessing the behavioural effects of teratogens have yet to be defined. Obviously such studies will play an increasing role in teratological testing in the future for, as has recently been stated, "the social and political implications of such a situation are enormous"¹³.

Additional studies are in progress to re-examine Kerr's Pink blighted potato, and the effects of cytochalasin and solanine on marmoset morphogenesis.

We thank Dr Barbara Lund and G. Lyon for providing potatoes infected with *Erwinia carotovora*.

D. E. POSWILLO
D. SOPHER
S. J. MITCHELL

Royal College of Surgeons of England,
Research Establishment,
Downe, Orpington, Kent

D. T. COXON
R. F. CURTIS
K. R. PRICE

Chemistry Division,
Agricultural Research Council,
Food Research Institute,
Colney Lane,
Norwich NOR 70F

Received April 13, 1973.

- ¹ Poswillo, D. E., Sopher, D., and Mitchell, S., *Nature*, **239**, 462 (1972).
- ² Renwick, J. H., *Br. J. prev. soc. Med.*, **26**, 67 (1972).
- ³ Baker, L. C., Lampitt, L. H., and Meredith, O. B., *J. Sci. Food Agric.*, **6**, 197 (1955).
- ⁴ Wierzchowski, P., and Wierschowska, Z., *Chem. Anal. (Warsaw)*, **6**, 579 (1961).
- ⁵ Lyon, G. D., *Physiol. Plant Pathol.*, **2**, 411 (1972).
- ⁶ Spyker, J. M., and Smithberg, M., *Teratology*, **5**, 181 (1972).
- ⁷ Shepard, T. H., *Lancet*, **i**, 96 (1973).
- ⁸ Linville, G. P., and Shepard, T. H., *Nature new Biol.*, **236**, 246 (1972).
- ⁹ Carter, S. B., *Nature*, **213**, 261 (1967).
- ¹⁰ Hide, G. A., Hirst, J. M., and Griffith, R. L., *Proc. Fifth. Br. Insect. Fungic. Conf.*, 310 (1969).
- ¹¹ Wilson, G. S., *Mon. Bull. Min. Health*, **18**, 207 (1959).
- ¹² Kline, B. E., von Elbe, H., Dahle, N. A., and Kupchan, S. M., *Proc. Soc. exp. Biol. Med.*, **107**, 807 (1961).
- ¹³ Leading article, *Lancet*, **ii**, 1349 (1972).

mating ability of released males¹. In the case of *Aedes aegypti*, these conclusions were experimentally confirmed by Weidhaas and Schmidt². Adults emerged from male pupae irradiated at 8,000 and 10,000 r inseminated only 4 and 0% of the females respectively when released into laboratory populations at a ratio of four treated males to one untreated.

It has been observed that the effects of irradiation on living organisms may be modified by changing the internal environment of the cell experimentally. Thoday and Read³ decreased the oxygen concentration in irradiated *Vicia faba* and found a lower mutation frequency. Hollander, Baker and Anderson⁴, in a study of the mutation rate of sex-linked lethals induced by irradiation in male *Drosophila melanogaster*, found the mutation rate to vary from 8 to 16% correlative with increased oxygen concentration. Baldwin and Chance⁵ reported on the improved competitiveness of males of *Rhodnius prolixus* sterilized by irradiation in the absence of oxygen. Nitrogen-treated males lived longer than air-irradiated males and competed successfully for females with untreated males. Hooper⁶ demonstrated, at the 98% level of male sterility in the Mediterranean fruit fly, *Ceratitis capitata*, that irradiation in nitrogen resulted in males which were three times more competitive than males irradiated in air.

In order to bypass the debilitating effect of radiation and to produce competitive sterile males, we investigated the role of irradiation in nitrogen in induced sterility in *Aedes aegypti*. Male pupae and 1-d-old adults were irradiated at 3,500, 7,000 and 10,000 r, respectively, either in air or in nitrogen and their competitive mating ability was tested against untreated males. Nitrogen was allowed to flow through glass tubes containing pupae and 1-d-old males. The chambers were clamped off and the "anaesthetized" condition of the mosquitoes was used as an indicator of the absence of oxygen. The males were then allowed to acclimatize themselves to the nitrogen environment for 0.5 h before exposure to radiation. Chambers containing males in a nitrogen environment and males in an air environment were exposed to gamma irradiation at the three doses. Two days after exposure, experimental and untreated males were released simultaneously into colony cages containing 4-d-old virgin females. The ratio of the mosquitoes in each cage was one treated male to one untreated male to one untreated female. Each replicate contained fifty treated males, fifty untreated males and fifty untreated females. Females were blood fed and egged separately. The lack of hatchability or high egg hatch were used as indicators of whether a female was inseminated by a sterile or a normal male respectively.

Table 1 gives the average hatchabilities of females inseminated by control and irradiated males and indicates that hatchability is a valid determination of whether an irradiated or control male inseminated a given female.

Table 1 Average % Hatchability of Egg Papers after Irradiation of Inseminating Males

	Unirradiated (control)	3,500 rad	7,000 rad	10,000 rad
In air	96	30	0	0
In nitrogen	95	36	16	0

Radiation Sterilization of *Aedes aegypti* in Nitrogen and Implications for Sterile Male Technique

ONE of the problems that often arise with mosquitoes and other insects when radiation-induced dominant lethality (sterile-male technique) is used is that sterilized males cannot compete with untreated native males for matings with females. It is generally believed that the field trials of releases of radiation-sterilized males of *Aedes aegypti* in the United States were unsuccessful because radiation impaired vigour and competitive

The data in Table 2 demonstrate that, in the conditions of this experiment, males irradiated to 7,000–10,000 r dose in the presence of nitrogen were as competitive as normal males in mating with females, whereas air-irradiated males were not. Moreover, males exposed to a low dose of gamma radiation whether in air or nitrogen seemed to be three to four times as competitive as non-irradiated males. These results indicate that nitrogen may protect males sterilized by irradiation sufficiently to permit the sterile male technique to be effective in the control of *Aedes aegypti* and other insect species.

Table 2 % of Females Inseminated by Treated Males

Replicate No.	Irradiation in air	Irradiation in nitrogen
	Stage irradiated—male adults (3,500 r)	
	80 (20)*	70 (30)*
	Stage irradiated—male pupae (7,000 r)	
1	26 (74)	55 (45)
2	12 (88)	51 (49)
3	18 (82)	46 (44)
Average	18 (82)	50 (50)
	Stage irradiated—male adults (7,000 r)	
1	11 (89)	53 (47)
2	20 (80)	47 (53)
3	20 (80)	55 (45)
Average	17 (83)	51.6 (48.4)
	Stage irradiated—male adults (10,000 r)	
1	24 (76)	55 (45)
2	13 (87)	52 (48)
3	18 (82)	51 (49)
Average	18.3 (81.7)	52.6 (47.4)

* Figures in parentheses indicate % females inseminated by untreated males.

This work received support from an Atomic Energy Commission contract with the Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana.

E. HALLINAN
K. S. RAI

Department of Biology,
University of Notre Dame,
Notre Dame, Indiana 46556

Received April 295, 1973.

¹ Morlan, H. B., McCray, jun., E. M., and Kilpatrick, J. W., *Mosq. News*, 22, 295 (1962).

- ² Weidhaas, D. E., and Schmidt, C. H., *Mosq. News*, 23, 32 (1963).
³ Thoday, J. M., and Read, J., *Nature*, 160, 608 (1947).
⁴ Hollander, A., Baker, G., and Anderson, H., *Cold Spring Harb. Symp. Quant. Biol.*, 16, 315 (1951).
⁵ Baldwin, W. F., and Chance, G. D., *IAEA SM/138/1* (1970).
⁶ Hooper, G. H. S., *J. econ. Ent.*, 64, 1364 (1972).

New Complexes of Phenols and Lactams

COMPLEXES of drugs, including phenols, are of continuing interest, especially to pharmacologists, for purposes of solubilization, administration, antagonism, detoxification and controlled release¹⁻¹³. Schmulbach *et al.*¹⁴ studied the spectrography of phenols complexed with amides in solution but no products were isolated or characterized, only a 1:1 complex was suggested. Although polyvinylpyrrolidone (PVP) is known to form complexes with phenols^{1-3,5,7}, these have not been well defined because of molecular weight variation and steric inhibition of complete complexation.

We have now found a number of phenols which form well defined, stable, crystalline complexes with several lactams. Our N-substituted lactams (pyrrolidones) formed the complexes in the ratio of one amide to two phenolic hydroxyls. The lactams unsubstituted on the nitrogen (2-pyrrolidone, ϵ -caprolactam) formed the complexes in the ratio of one lactam to one hydroxyl. We were not able to isolate complexes involving phenols with substituents out of the plane of the ring such as *p*-nitro-, *p*-amino-, *p*-phenyl-phenol or 1-hydroxy-anthraquinone. Polymeric complexes from polyfunctional starting materials were not observed.

The complexes were prepared by mixing the phenol with the lactam in the correct stoichiometric proportion, heating until liquid, then cooling (see legend to Table 1). Alterna-

Table 1 Summary of Complex Experimentation

A	Complex components B	A : B (molar)	Reaction temp (°C)	Melting point, uncorrected (°C)	Recrystallization solvent
* HMBP	Phenol	1 : 4	50	54-56	Benzene
† HMBP	Hydroquinone	1 : 2	160	133-135	Water
‡ NMP	Hydroquinone	1 : 1	135	95-98	Toluene
		1 : 1	Boil	96-98.5	None
NMP	2,4,5-Trichlorophenol	1 : 1	90	62-64	Heptane
§ CHP	Hydroquinone	1 : 1	80	52-57	None
Pyrrolidone	Hydroquinone	2 : 1	130	135-138	Water
			130	130-132	Toluene
Pyrrolidone	Phenol	1 : 1	50	28-30	None
Caprolactam	Hydroquinone	2 : 1	130	117-119	Toluene
				117-119	Water
				116-119	None
Pyrrolidone	2,4,5-Trichlorophenol	1 : 1	90	73-77	Heptane
Caprolactam	Phenol	1 : 1	70	34-36	Heptane
HMBP	3,4-Dichlorophenol	1 : 4	85	54-57	Toluene
HMBP	2,4,5-Trichlorophenol	1 : 4	85	82-85	Heptane-toluene
HMBP	2,2'-Methylene-bis(4,6-dichlorophenol)	1 : 2	135	95-97	Heptane-toluene
HMBP	2,2'-Methylene-bis(3,4,6-trichlorophenol)	1 : 2	120	116-117	Cyclohexane-toluene

Most of the phenolics were regular stock items from chemical suppliers; better grades were used as received. All the pyrrolidones were distilled. These were prepared by well known methods¹⁵ from ammonia or amines and γ -butyrolactone as in the following example. A mixture of 604 g (5.2 mol) of hexamethylenediamine and 896 g (10.4 mol) of γ -butyrolactone was heated in an autoclave at 270° C for 7 h. The product was distilled using a Vigreux column. After stripping off the water of reaction, the N,N'-hexamethylene-bis-(2-pyrrolidone) (HMBP) distilled at 215° C at 0.2 mm Hg in 96% yield. The HMBP was soluble in water; n_D^{20} = 1.5032 (ref. 16). Except as noted, the complexes were prepared without solvent; solvents were used at first to discover combining ratios. Infrared curves showed that many bands of the starting materials persisted in the complexes but some were substantially shifted.

* HMBP (1 mol) and phenol (4 mol) (thus one amide to two phenolic hydroxyls) were combined at 50° C. Slight warming occurred immediately. A hygroscopic crystalline mass formed on cooling. Recrystallized from benzene, melting point, 54-56° C. Calculated for $C_{38}H_{48}N_2O_6$: N, 4.46%; found 4.26%.

† The complex was prepared neat, as tabulated; also by combining aqueous solutions. It was prepared also by combining ammoniacal HMBP with aqueous hydroquinone. For the recrystallized complex were calculated $C_{26}H_{36}N_2O_4$: C, 66.1%; H, 7.69%; N, 5.94%. Found were C, 66.12%, 66.27%; H, 7.65%, 7.73%; N, 5.96%. Infrared band shifts were observed.

‡ NMP, N-methyl-2-pyrrolidone.

§ CHP, N-cyclohexyl-2-pyrrolidone. The product was chilled to 0° C to initiate crystallization.

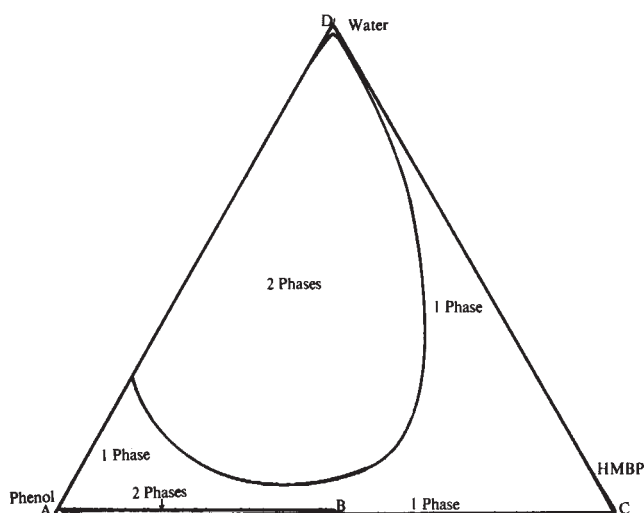


Fig. 1 The water-phenol-hexamethylene-bis-(2-pyrrolidone) ternary system at 29° C. The curved line was found by taking known solutions of two of the components in a stirred Erlenmeyer flask and, with the temperature at $29 \pm 0.5^\circ$ C, titrating in the third component until the number of phases changed. The line was approached from either side and the ends were derived from the literature¹⁷. The line AB, displaced from the edge of the chart for visibility, was determined by titrating HMBP into phenol, any small second dimension being ignored.

tively, the complex often precipitated from admixed solutions of the starting materials. Crystallization was sometimes successful from water and usually successful from neutral organic solvents as benzene.

The complexes were of constant composition regardless of starting composition and were crystals with sharp melting points. The N,N'-hexamethylene-bis-(2 pyrrolidone) (HMBP) (melting point 26° C) complex with phenol (melting point 41° C) melted at 54–56° C. The formation of the complex was exothermic. Altered spectral (infrared) and physiological (germicidal) properties were observed.

The complexes were thermally stable at least to their melting points. They were stable in many organic solvents and many were stable in water. The HMBP-hydroquinone complex was stable and could be formed in a series of liquids of various basicity, including aqueous ammonium hydroxide, pyridine, styrene, toluene, benzene, water, dioxane, acetic acid and monochloroacetic acid. This complex could not be formed, and was decomposed in the presence of piperidine to give the piperidine salt of hydroquinone. Also it could not be formed and was decomposed by the presence of benzoquinone, the hydroquinone appearing as quinhydrone. The HMBP-phenol complex was evidently stable in water because we observed a substantially reduced toxicity to *S. aureus*. The phase relationships in this ternary system are shown in Fig. 1.

The exact nature of the binding forces in the complex has not been elucidated. On the basis of information now available we propose a plane-to-plane charge transfer stacking coupled with hydrogen bonding.

D. I. RANDALL
E. M. SMOLIN
J. P. COPES

GAF Corporation,
1361 Alps Road,
Wayne, New Jersey 07470

Received March 16; revised June 7, 1973.

- ¹ Guttman, D. E., and Higuchi, H. J., *J. Am. Pharm. Ass.*, **55** (10), 659 (1956).
- ² Walker, G. C., *Ontario Coll. Pharm.*, **11** (1), 3 (1962).
- ³ Higuchi, T., and Kuramoto, R., *J. Am. pharm. Ass.*, **43** (7), 398 (1954).

- ⁴ Higuchi, T., and Kristiansen, H., *J. pharm. Sci.*, **59** (11), 1601 (1970).
- ⁵ Kabadi, B. N., and Hammarlund, E. R., *J. pharm. Sci.*, **55** (10), 1069 (1966).
- ⁶ Joesten, M. D., and Drago, R. S., *J. Am. chem. Soc.*, **84**, 2037 (1962).
- ⁷ Joesten, M. D., and Drago, R. S., *J. Am. chem. Soc.*, **84**, 2696 (1962).
- ⁸ *Remington's Practice of Pharmacy* (twelfth ed.), 1780 (Mack Printing, Easton, Pennsylvania, 1961).
- ⁹ Mirkovic, S., *Ark. Farm.*, **18** (3), 181 (1968) (in Croat).
- ¹⁰ Speiser, P., *Sci. Pharm.*, **37** (1), 14 (1969) (in German).
- ¹¹ Sugimoto, I., *Chem. Pharm. Bull.* (Tokyo), **16** (8), 1527 (1968) (in English), 94357j (1968).
- ¹² Barker, H., *Aust. J. Pharm.*, **49** (851), 553 (1968).
- ¹³ Toth, T., *Gyogyszereszet*, **11** (5), 166 (in Hungarian).
- ¹⁴ Schmulbach, C., and Hart, D., *J. org. Chem.*, **29**, 3122 (1964).
- ¹⁵ Reppe, W., et al., *Ann. Chem.*, **596**, 1 (1955).
- ¹⁶ Haarer, E., and Fuerst, E., to *Badische PG 1*, **152**, 777 (1963).
- ¹⁷ Hougen, O. A., and Watson, R. M., *Chemical Process Principles Combined*, **142**, **1**, **2**, **3** (Wiley, New York, 1948).

Chemosensory Mechanism in American Cockroach Olfaction and Gustation

ALTHOUGH numerous theories of olfaction and gustation^{1–8} have been published, there is little experimental evidence on the energy-transfer mechanisms involved in the interactions of chemical messengers with proteinaceous receptors of sensory neurones. One such mechanism, involved in the inhibition of feeding of *Periplaneta americana* and *Scolytus multistriatus* by various 1,4-naphthoquinones, has been extensively studied and partially interpreted behaviourally^{9–16}, ultrastructurally^{10–16}, electrophysiologically^{10–16} and biochemically^{10–16}. These messenger naphthoquinones were shown to act through a selective reaction with sulphhydryl^{10–16} groups in a particular membrane-associated receptor macromolecule which was proven to be physically accessible *in situ*¹⁷ to these chemicals through cuticular pores and tubules^{10–16} in the walls of chemoreceptor sensillae on the insect's antennae. This specific energy transfer resulted in disulphide-bond formation or thiol complexing^{10–16}, both of which are proven¹⁸ mechanisms in certain proteins for causing conformational changes. Such changes can alter the cationic permeability of macromolecules composing membranes¹⁸. The messenger-altered cation permeability of the receptor thus can result in the generation of action potentials in the sensory neurone^{10–16}. Here we report certain physicochemical characteristics of the messenger-naphthoquinone receptor protein interactions as analysed in a deoxygenated system at physiological pH by the *in vitro* technique of dropping mercury electrode (DME) polarography (PO4 'Polariter', London Co.). The polarogram is a current-voltage curve obtained by applying a gradually increasing voltage to a system and measuring the current flow at each potential. Characteristic polarographic half-wave potential (that is, E 1/2) values for proteins were attributed exclusively to exposed, reactive sulphhydryl, or reducible disulphide, groups^{19–22}. In our own investigations, the 'Triton' X-100-solubilized quinone-reactive receptor protein from the cockroach antennae gave a characteristic E 1/2 of –1,860 mV. A control (non-quinone receptor) protein system composed of the saline-soluble proteins from the same antennae gave an E 1/2 of –1,800 mV. Adding messenger 5-hydroxy-1,4-naphthoquinone (5×10^{-5} M) in the receptor preparation characteristically shifted the E 1/2 from –1,860 to –1,880 mV; another less repellent, or feeding-inhibitory, messenger, 2-methyl-1,4-naphthoquinone (5×10^{-5} M), only shifted the receptor E 1/2 to –1,875 mV. Neither of these messenger quinones altered the –1,800 mV E 1/2 of the non-receptor control protein. N-Ethyl maleimide (NEM), a highly specific thiol reagent, at 5×10^{-5} M in the receptor preparation shifted the E 1/2 from –1,860 to –1,890 mV. In this –1,890 mV

state, the receptor was non-reactive with messenger quinones. Treatment of the receptor in the $-1,890$ mV state with the disulphide reducing agent, dithiothreitol (DTT), brought the E 1/2 of the receptor back down to $-1,860$ mV. In this restored $-1,860$ mV state, the receptor again reacted with messenger quinones.

The receptor protein was isolated from the antennae of adult male *P. americana* using the techniques of Ferkovich and Norris¹⁷. Two saline (0.9% NaCl), and then two (Triton) X-100 in 0.9% NaCl, extracts were thus made from 100 pairs of antennae which had been excised proximally and immediately dropped into cold saline. Protein concentrations were determined by the method of Lowry *et al.*²³ using crystalline bovine serum albumin as a standard.

In the polarographic analyses, the sample protein was diluted to $100 \mu\text{g ml}^{-1}$ of a potassium phosphate (0.1 M), pH 7.0, buffer which contained 25% (v/v) of 95% ethanol. The polarographic sample was deoxygenated with nitrogen before and during the analysis. Receptor samples (E 1/2 = $-1,860$ mV) left exposed to atmospheric conditions on the laboratory bench for 24 h became non-reactive to quinone messengers, and then had E 1/2 values of $-1,890$ to $-1,900$ mV. Ascorbic acid (0.01 M in the receptor-buffer preparation) maintained the receptor in its reactive (that is, $-1,860$ mV) state for up to 72 h.

These results thus provide evidence that the energy-transfer mechanism for messenger quinone-receptor interactions in these chemosensory nerves involves characteristic shifts in the $-\text{SH}/\text{S}-\text{S}-$ equilibrium in the receptor macromolecules. Such a mechanism of predictable macromolecular reorientation in nerve membranes in response to specific messengers can bring about alterations of inorganic ion flows characteristic of nervous excitation and impulse conduction. A natural system with variable sensitivities, such as now reported here, was postulated by Norris²⁴ as one basic mechanism for short- and long-term alterations of nerve membrane macromolecule state to binding functions²⁵. We believe learning and memory probably involve this fundamental mechanism.

This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison.

JACK M. ROZENTAL
DALE M. NORRIS

Department of Entomology,
University of Wisconsin, Madison

Received June 18, 1973.

- ¹ Moncrieff, R. W., *The Chemical Senses*, 760 (CRS Press, Cleveland, 1967).
- ² Hornstein, I., *Flavor Chemistry, Advances in Chemistry Series 56*, 278 (American Chemical Society, Washington, 1966).
- ³ Schultz, H. W., Day, E. A., and Libbey, L. M., *Symposium on Foods: The Chemistry and Physiology of Flavors*, 552 (AVI Publishing Co., Westport, Connecticut, 1967).
- ⁴ Davies, J. T., *J. theor. Biol.*, **8**, 1 (1965).
- ⁵ Dravnieks, A., *Nature*, **194**, 245 (1962).
- ⁶ Amore, J. E., *Cold Spring Harbor Symp. quant. Biol.*, **30**, 623 (1965).
- ⁷ Beidler, L. M., *Ann. NY Acad. Sci.*, **58**, 52 (1954).
- ⁸ Wright, R. H., *Nature*, **173**, 831 (1954).
- ⁹ Norris, D. M., Ferkovich, S. M., Rozental, J. M., Baker, J. E., and Borg, T. K., *Science*, **170**, 754 (1970).
- ¹⁰ Norris, D. M., Baker, J. E., Borg, T. K., Ferkovich, S. M., and Rozental, J. M., *Contrib. Boyce Thomp. Inst.*, **24**, 263 (1970).
- ¹¹ Norris, D. M., *Nature*, **222**, 1263 (1969).
- ¹² Norris, D. M., Ferkovich, S. M., Baker, J. E., Rozental, J. M., and Borg, T. K., *J. Insect Physiol.*, **17**, 85 (1971).
- ¹³ Borg, T. K., and Norris, D. M., *Ann. ent. Soc. Am.*, **64**, 544 (1971).
- ¹⁴ Ferkovich, S. M., and Norris, D. M., *Chem. Biol. Interactions*, **4**, 23 (1971/1972).
- ¹⁵ Baker, J. E., and Norris, D. M., *J. Insect Physiol.*, **17**, 2383 (1971).
- ¹⁶ Baker, J. E., and Norris, D. M., *Experientia*, **28**, 31 (1972).
- ¹⁷ Ferkovich, S. M., and Norris, D. M., *Experientia*, **28**, 978 (1972).
- ¹⁸ Rothstein, A., in *Current Topics in Membranes and Transport*, 243 (Academic Press, London and New York, 1970).

- ¹⁹ Brdička, R., *Coll. Czech. chem. Comm.*, **5**, 112 (1933).
- ²⁰ Brdička, R., *Coll. Czech. chem. Comm.*, **5**, 148 (1933).
- ²¹ Brdička, R., *Coll. Czech. chem. Comm.*, **5**, 238 (1933).
- ²² Brdička, R., *Coll. Czech. chem. Comm.*, **8**, 366 (1936).
- ²³ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
- ²⁴ Norris, D. M., *Experientia*, **27**, 531 (1971).
- ²⁵ Changeaux, J. P., and Thiéry, J., in *Regulatory Functions of Biological Membranes*, 311 (Elsevier, Amsterdam, 1968).

Ranking Characters by a Dispersion Criterion

It is often proposed by taxonomists and ecologists that classifications and other related analyses should be based on the possible largest number of characters. They believe that when large character sets are used the analysis will gain in accuracy, and the results will be more broadly applicable. Yet sampling, computational and other logistic problems are often associated with the handling of large character sets. Some characters may have to be deleted in the interest of more economical sampling and computations.

Appropriate criteria must be found to evaluate an individual character's relative importance. I show that relative importance can be measured experimentally, based on a character's independent share of the total dispersion in a pilot sample of individuals, and the rank order of the importance values can be used to select those characters worth further investigation.

I consider a pilot sample of N individuals described on the basis of p characters and with sample structure Q_{Np} . I assume that the p characters represent a sub-sample of the total number of available characters P , the sample structure of which in the N individuals is Q_{Np} . I derive $\sigma(P, p)$ as a measure of the stress (or distortion) in Q_{Np} relative to Q_{NP} . One may visualize Q_{Np} and Q_{NP} as $N \times N$ symmetric matrices which define the pairwise resemblance of the N individuals on the basis of their characters. The elements may represent any kind of symmetric measures.

When the goal is to reduce the number of characters, one may select them for deletion so that the stress function is minimized for any value of p ; equivalently, the structure Q_{Np} will represent a best possible approximation to Q_{NP} . The total number of distinct p samples that can be drawn from a P sample is $P!/(p!(P-p)!)$. One could proceed with the selection of the best p sample as follows: Extract all distinct p samples; compute $\sigma(P, p)$ for each; and select the p sample for which $\sigma(P, p)$ is minimum. For varying values of p , the results will provide a basis for the selection of those characters which should be considered further.

There is one obvious defect in this procedure: the number of discrete p samples may be unmanageably large. A computationally simpler method is needed. Such a method may use the rank order of characters determined on the basis of a dispersion criterion given by the sum of squares (or some related measure). The steps include: (1) Designate a data matrix by X , and its elements by X_{hj} . The subscripts identify respectively a character (h) and an individual (j). There are P characters (row vectors) and N individuals (column vectors). (2) Centre the data within characters, as shown in equation (1), to obtain a new data matrix A with elements A_{hj} . (In equation (1), $\bar{X}_h$ represents the arithmetic mean of observations for character h . The data should be standardized if the characters used are not commensurable). (3) Compute a $P \times P$ dispersion matrix R for the characters on the basis of equation (2). (If the characters were standardized, R would represent a correlation matrix. An element of R is r_{hi} relating character h and character i .) (4) Compute the dispersion criterion S_i (given by equation (3)) to identify character m the rank order of which is one. (For computation of S_i , the value of subscript e is set at 1. Character m has maximum dispersion in the P sample.) (5) Determine the character whose rank order is two, and rank all the remaining characters on the basis of criterion $S_2, \dots, S_k, \dots$. In each

Table 1 Sample Data

		Individuals																	Mean $\bar{X}_h$
Characters		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
		Lengths (0.1 mm units)																	
Corolla	(X_1)	75	61	53	77	41	56	60	57	48	53	58	49	40	60	66	47	50	55.9
Calyx	(X_2)	27	17	20	19	16	18	19	28	29	35	37	29	30	36	45	36	41	28.4
Style	(X_3)	65	54	64	57	33	62	64	46	53	54	54	64	31	48	39	31	30	49.9
Corolla lobe	(X_4)	11	11	13	15	12	19	21	11	10	8	10	13	4	9	7	4	4	10.7
Pedicel*	(X_5)	15	16	13	12	11	14	21	17	16	14	18	26	6	13	16	10	7	14.4

* In 1 mm units.

The entries in the table represent averages of ten independent measurements, 1-7 *Phyllodoce empetrifloris* (Sm.) D.; 8-12 *Phyllodoce × intermedia* (Hook.) Camp; and 13-17 *Phyllodoce glanduliflora* (Hook.) Coville. The sample represents part of a larger collection presently under study in this department.

case, the dispersion criterion is computed from a new residual R , in which the effect of those characters which were already ranked is removed from the correlations. A residual element of R is defined by

$$r'_{hi} = r_{hi} - y_{hm}y_{im}$$

where r_{hi} represents an original value in R , or some residual portion of the original value, and y_{hm} and y_{im} are coordinates,

$$y_{hm} = r_{hm} / \sqrt{r_{mm}}$$

$$y_{im} = r_{im} / \sqrt{r_{mm}}$$

The subscript m identifies the character whose rank order has just been determined.

$$A_{hj} = X_{hj} - \bar{X}_h \quad (1)$$

$$r_{hi} = \sum A_{hj}A_{ij}, j = 1, \dots, N \quad (2)$$

$$S_e = \max \left[\sum_{h=1}^P r_{h1}^2 / r_{11}, \dots, \sum_{h=1}^P r_{hP}^2 / r_{PP} \right] \quad (3)$$

This ranking is based on each character's independent share (S_e) of the total dispersions given by the sum of the diagonal elements in R . The best, or nearly the best, p sample is formed from the p highest ranking characters.

S_e or $100 S_e / \sum_{h=1}^P r_{hh}$ may be used as a measure of sample

weight, or "importance" value, for the characters. But such a measure of importance will represent a meaningful input from a pilot study for further consideration in those analyses which are constrained because: the r_{hi} represent meaningful spatial parameters in the sample space of the intended analysis; the intended analysis is performed on the same set of p characters; and the possibility exists to expand the character set to the same P characters from which the subset of p characters were originally drawn.

When the r_{hi} are defined as sum of squares and cross products, the ranking by this method will represent valid input, for instance in sum of squares clustering¹, ordinations² which use Euclidean distance or vector scalar products, and methods which operate within the framework of an appropriate Euclidean sample space. A quantity similar to S_e , but excluding self-comparisons, has been used³ in classificatory analyses based on ranking.

The ranking method is illustrated in Table 1. The R matrix is defined as a correlation matrix (to remove the effect of scale differences of the characters from the analysis) given in Table 2. From these, I compute $S_1 = \max (1.4429, 1.7641, 2.4983, 2.5301, 1.8457) = 2.5301$ corresponding to $m=4$. Rank 1 thus is assigned to character 4. This is the character which accounts for a maximum portion of the total dispersion in the 17 plant sample. (Numerical results are given within computer rounding errors.)

The first residual of R , after removing the effect of character 4 from correlations, is given in Table 3. The zero row and column are retained in this table to make it easier to keep track of those

Table 2 Upper Half of the Correlation Matrix R for Five Characters Listed in Table 1

1.0000	-0.0622	0.5076	0.3467	0.2475
	1.0000	-0.4577	-0.7310	-0.1281
		1.0000	0.7546	0.6796
			1.0000	0.5533
				1.0000

Table 3 The First Residual of R Obtained by Removing the Effect of Character 4 from the Correlations Given in Table 2

0.8798	0.1913	0.2460	0.0000	0.0557
	0.4656	0.0940	0.0000	0.2764
		0.4306	0.0000	0.2621
			0.0000	0.0000
				0.6939

characters which are still remaining in the analysis. From the first residual correlations I obtain $S_2 = \max (0.9937, 0.7272, 0.7512, 0.0000, 0.9075) = 0.9937$ and $m=1$. This establishes 2 as the rank order for character 1.

Similarly, $S_3 = \max (0.0000, 0.5927, 0.5344, 0.0000, 0.8796) = 0.8796$ and $m=5$, so the rank order for character 5 is 3, and $S_4 = \max (0.0000, 0.3318, 0.2844, 0.0000, 0.0000) = 0.3318$, corresponding to $m=2$. The rank order for character 2 is 4, and 5 for character 3. In the last residual of R there is only one non-zero value (0.2648) located in the third diagonal cell.

The results are summarized in Table 4. The total of the S_e values is 5, the same as the sum of the diagonal elements in the correlation matrix. This implies that the ranking is based on truly orthogonal partitions.

Table 4 Character Ranks and Other Ranking Statistics

Character in Table 1	Rank order (e)	Character's specific dispersion (S_e)	Relative importance ($100 S_e / \sum r_{hh}$)
X_1	2	0.9937	19.9
X_2	4	0.3318	6.6
X_3	5	0.2648	5.3
X_4	1	2.5301	50.6
X_5	3	0.8796	17.6
Totals		5.0000	100.0

The problem of identifying a cutoff point in the rank order, below which no character will be further investigated, will arise. Ideally, the decision should be based on some statistical criterion which could facilitate a test for the hypothesis that the last k lowest ranking characters do not contribute significantly to dispersion in the sample. Because I know of no such test, I suggest the use of an alternative method for determining the

cutoff point: omit the last q characters whose importance value is zero or nearly so; compute the stress curve $\sigma(P, p)$ at fixed P and varying p representing samples of the first $P-q$, $P-q-1$, $P-q-2$, . . . highest ranking characters; inspect the stress curve and select a p sample in the region where the curve starts flattening off.

The research was supported by a grant from the National Research Council of Canada.

LASZLO ORLOCI*

Department of Botany,
College of Arts and Sciences,
University of Hawaii

* Present address: Department of Plant Sciences, University of Western Ontario, London 72, Ontario, Canada.

Received April 14; revised August 29, 1972.

¹ Ward, J. H., *J. Am. Statist. Ass.*, **58**, 236 (1963).

² Orloci, L., *Handbook of Vegetation Science* (edit. by Tüxen, R.), **5** (Junk, The Hague, 1972).

³ Williams, W. T., and Lambert, J. M., *J. Ecol.*, **47**, 83 (1959).

Multivariate Analysis of the *Dryopithecus africanus* Forelimb

CORRECT interpretation of fossil postcranial remains is crucial to understanding primate phylogeny. This is very apparent in the study of the postcranial remains of the East African Early Miocene hominoid *Dryopithecus africanus*¹. In 1951 Whitworth discovered a nearly complete forelimb from the Gumba Peninsula, Rusinga Island, Kenya, as part of an association with dental and cranial remains of *D. africanus*. The deposits date from 19 to 16 m.y.^{2,3}. Napier and Davies³ concluded that the forelimb of *D. africanus* showed many features reminiscent of monkeys, for example, colobines and atelines, which supposedly use their forelimbs alone for locomotion a significant amount of the time. They further claimed that "in the absence of *a priori* evidence as to the probable nature of the fossil remains provided by the associated jaw fragments, it is to be doubted whether there would have been sufficient evidence from the forelimb bones alone to postulate the pongid affinities of *D. africanus* . . .". This view has gained wide acceptance⁴⁻⁶.

Because many shared postcranial features of *Pan* and *Gorilla* were supposedly not present in *D. africanus*, two alternative hypotheses concerning the *Pan*-*Gorilla* (and basal hominid) radiation were proposed: (1) that the knuckle-walking adaptations of both *Pan* and *Gorilla* represent a case of extensive parallel postcranial evolution since the Early Miocene⁷; or (2) that the *Pan*-*Gorilla* radiation must have occurred more recently than the Miocene⁸⁻¹⁰.

Following Napier and Davis³, it has been generally assumed that *D. africanus* was postcranially most similar to the so-called "semi-brachiating" monkeys like *Presbytis* and *Colobus*^{4,6}. These two forms are active arboreal palmigrade quadrupeds. Recent re-examinations of the forelimb anatomy of *D. africanus* indicate that the view of *D. africanus* as merely a "dental ape" might be in error. Many of the anatomical features of the *D. africanus* forelimb most closely resemble *Pan*, and are markedly non-cercopithecoid-like (refs. 10 and 11, and personal communication from A. C. Walker).

Deperet¹³, in 1887, was the first to put forward the view, later often supported in the writings of Le Gros Clark⁶, that postcranial bones of Tertiary apes resemble in various ways the same bones of living monkeys rather than those of hominoids: the *scala naturae*. Naturally, Miocene apes do

share some structural features with monkeys that are not now seen in hominoids, but many of these primitive features are also found in lemurs and lorises, and thus can hardly be termed exclusively "monkey-like". For these and other reasons, the forelimb similarities of *D. africanus* to cercopithecoid and ceboid monkeys seem to have been over-emphasized.

Multivariate statistical analysis provides an effective way of establishing affinities between fossil and living primates^{14,15}. We have used the multivariate techniques of principal components and principal coordinates analysis, which do not necessitate previous categorization of specimens into groups^{16,17}.

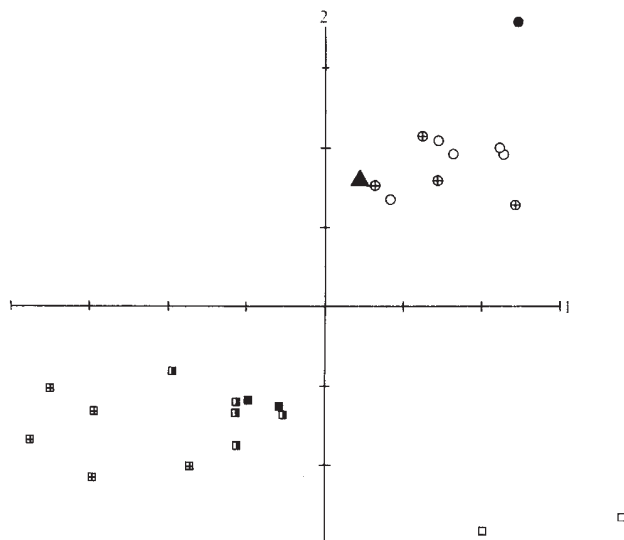


Fig. 1 Plot of the principal component scores of primate forelimbs along the first and second principal component axes, respectively subsuming 71.8 and 17.8% of the total variance. $\blacktriangle$, *Dryopithecus africanus*; $\oplus$, *Pan paniscus*; $\circ$, *Pan troglodytes*; $\bullet$, *Gorilla gorilla*; $\blacksquare$, *Presbytis* sp.; $\triangle$, *Papio* sp.; $\square$, *Cercopithecus* sp.; $\square$, *Ateles* sp.

To establish the locomotor affinities of *D. africanus*, we performed components and coordinates analysis using sixteen indices of the forelimb listed originally by Napier and Davis³. Indices rather than raw measurements were used in order to minimize the significance of size differences (see Table 1 for indices used). Twenty-five forelimb specimens were included in the sample: one *D. africanus*, four *Pan paniscus*, one *Gorilla gorilla*, five *Pan troglodytes*, two *Ateles*, two *Presbytis*, five *Papio* and five *Cercopithecus*. All values are taken from Napier and Davis³ except for *Gorilla* and *Pan paniscus*, which we measured. *Pan paniscus* indices were included because it is the pongid closest in size to *D. africanus*; this reduces the disruptive effects of allometric differences.

In Fig. 1, there is a clear separation between pongids and monkeys, as well as a separation between the spider monkey and all other monkeys. With respect to locomotion, the first component separates quadrupedal cercopithecoids from both the knuckle-walkers (*Pan* and *Gorilla*) and the quadrupedal arm-swingers (so-called "semi-brachiator") *Ateles*. The second component separates knuckle-walkers from all non-knuckle-walking quadrupeds. With respect to both of these axes, the forelimb of *D. africanus* falls among the apes, indicating that the total morphological pattern of the forelimb is pongid-like, not monkey-like. The remaining axes fail to separate apes from monkeys, and thus are not particularly relevant for our purposes. These results were verified by a principal coordinates analysis on the same forelimb measurements (see Fig. 2).

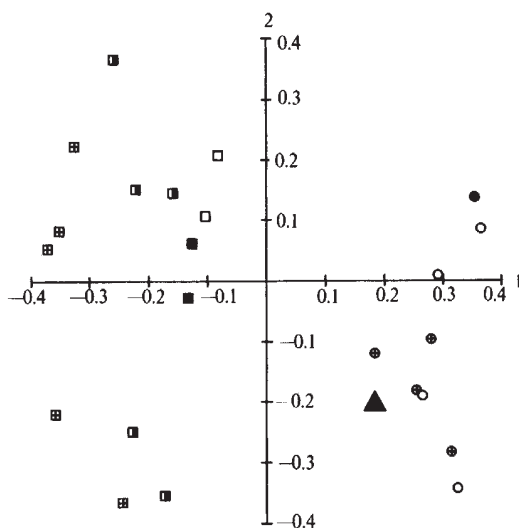


Fig. 2 Plot of the principal coordinate scores of primate forelimbs along the first two coordinate axes. Symbols as in Fig. 1.

The variates which contribute most to the separation seen in Fig. 1 can be determined by examining the weighting of each index in Table 1. The most important variates which affect the first two axes are indices 10, 8, 11, 9, 3 and 2. Because the heavy weightings of these indices are primarily responsible for both the separation between apes and monkeys, and the placement of *D. africanus* among the pongids, the functional significance of these indices should be assessed.

Table 1 The Indices Used in the Principal Components Analysis and their Weightings for the First Three Components

Index	First component	Second component	Third component
(1) Groove	0.001	-0.050	-0.086
(2) Deltoid	0.103	0.168	-0.098
(3) Deltoid-latissimus dorsi	0.136	0.207	-0.210
(4) Supinator crest	-0.059	-0.004	-0.249
(5) Bicipicondylar	0.017	0.117	0.038
(6) Articular width	0.001	0.076	-0.001
(7) Medial epicondyle	0.066	0.041	0.031
(8) Trochlear	0.195	0.786	0.465
(9) Brachial	-0.137	-0.224	0.047
(10) Radial neck	0.945	-0.282	0.012
(11) Ulnar styloid	-0.091	-0.385	0.798
(12) Relative metacarpal length	-0.004	0.053	0.040
(13) Relative phalangeal length	0.043	-0.059	-0.066
(14) Relative carpal length	-0.031	0.023	0.064
(15) Relative hand length	0.027	0.034	-0.073
(16) Relative humerus length	0.010	0.030	0.053
% of total variance	71.7	17.8	5.7

Indices are listed in order of appearance in Napier and Davis appendices I-IV, where they are defined³.

The most significant index on the first axis, which separates knuckle-walkers and the arm-swinging *Ateles* from palmigrade quadrupeds, is the radial neck index. In forms such as *Ateles* which use their forelimbs alone for propulsion for a significant part of their daily activities, the radial neck is relatively long, as it is in *Limnopithecus*¹⁸. Such an anatomical arrangement augments the power of the biceps brachii muscle around the elbow joint by increasing the lever arm/load arm

ratio of the radius. Powerful flexion and supination of the forearm are both necessary adaptations for animals which suspend and propel themselves by their forelimbs alone. In the pongids, who often climb by pulling themselves up with the forearms, the long radial neck is also important, although not as significant as in arm-swingers and brachiators (that is, gibbons).

Two other indices which are important contributors to separation along the first axis are concerned with the distal insertion of the deltoideus on the humerus. The low insertion of deltoideus characterizes pongids as opposed to cercopithecoids. This reflects the importance of the deltoideus in forms capable of powerful abduction of the forelimb.

On the second axis, the greatest discriminating index separating knuckle-walkers from all monkeys is the trochlear index. This index is also the second greatest contributor to the first component. A relatively high trochlear width in pongids is associated with high degrees of rotational ability in the forelimbs. In pongids, powerful forearm flexion is possible whether or not the hand is in the pronated or supinated position. The stability of the pongid elbow joint is maintained by the fit of the humerus and ulna (and by the ulnar collateral ligaments), regardless of the position of the radius. In monkeys, which are capable of only about 90° of supination, the proximal ulna is narrow (as is the corresponding trochlea of the humerus) and the radial head is oval in shape. Both of these bones are responsible for maintaining the integrity of the elbow joint in cercopithecoids⁸. Besides possessing a high trochlear index, *D. africanus* also has a rounded radial head.

The second most important index which contributes to the second axis of the principal components analysis is the ulnar styloid index, which is low in knuckle-walkers and high in monkeys and *D. africanus*. The length and morphology of the ulnar styloid process were considered by Napier and Davis³ to indicate that *D. africanus* had a monkey-like wrist joint. Lewis^{12,19}, however, has analysed the wrist joints of monkeys, apes and *D. africanus* and has stressed that several pongid features do appear in the *D. africanus* wrist joint. Lewis has suggested that this is an adaptation facilitating suspensory posture; however, these features of wrist morphology are more reminiscent of knuckle-walkers, especially *Pan*, than of brachiators, that is, gibbons.

The brachial index is also important in discriminating knuckle-walkers from monkeys along both axes in Fig. 1. The low brachial index in *D. africanus* suggests that it was not adapted for either fast digitigrade running or habitual arm-swinging.

It can be concluded that the forelimb of *D. africanus* is much more similar to pongids than to monkeys with respect to the sixteen indices devised by Napier and Davis³. The similarity of these indices in fossil and modern apes goes far to dispel the widely held assumption that Miocene apes only resemble modern apes in their dental anatomy. Whether or not *D. africanus* was a knuckle-walker is a question requiring further investigation, but from this analysis it can at least be seen that *D. africanus* was not a *Presbytis*-like quadruped, as has been suggested. Considering rates of evolution and the fact of mosaic evolution, an early Miocene possible ancestor of *Pan* should not be expected to have been identical to the modern chimpanzee postcranially. The strong implication is, however, that if *D. africanus* was not a knuckle-walker, it may have been in some ways pre-adapted to that form of locomotion. A possible model could be the hand posture of certain New World monkeys when walking on the ground. Weight is borne on the heel of the hand and the dorsum of the phalanges, which are "tucked under" (personal communication from M. Schön). There is no need to consider the modern postcranial and locomotor similarities of *Pan* and *Gorilla* as a puzzling case of parallelism. Instead, by Miocene times at least one species of fossil ape had developed a postcranial anatomy

foreshadowing that of *Pan* and *Gorilla*; this ape thus represents an ideal structural ancestor, not only dentally but also postcranially, for one or more of the modern knuckle-walking apes.

We thank D. Pilbeam, E. Simons, A. Walker, P. Andrews and M. Schön for their helpful and detailed comments and criticisms.

MIKE ZWELL
GLENN C. CONROY

Department of Anthropology,
Yale University,
New Haven, Connecticut 06520

Received July 24, 1972; revised April 30, 1973.

- ¹ Hopwood, A. T., *J. Linn. Soc.*, **38**, 437 (1933).
- ² Van Couvering, J. A., and Miller, J. A., *Nature*, **221**, 628 (1969).
- ³ Napier, J. R., and Davis, P. R., *Fossil Mammals of Africa*, No. 16 (British Museum (Natural History), London, 1959).
- ⁴ Campbell, B. G., *Human Evolution* (Aldine, Chicago, 1966).
- ⁵ Tuttle, R. H., *Science*, **166**, 953 (1969).
- ⁶ Clark, W. E. Le Gros, *Antecedents of Man* (Harper and Row, New York, 1959).
- ⁷ Simons, E. L., *Ann. NY Acad. Sci.*, **102**, 282 (1962).
- ⁸ Washburn, S. L., *Condon Lectures* (Oregon State System of Higher Education, 1968).
- ⁹ Sarich, V. M., in *Perspectives on Human Evolution*, 1 (edit. by Washburn, S. L., and Jay, P. C.) (Holt, Rinehart and Winston, New York, 1968).
- ¹⁰ Sarich, V. M., and Wilson, A. C., *Science*, **158**, 1200 (1968).
- ¹¹ Conroy, G. C., and Fleagle, J. G., *Nature*, **237**, 103 (1972).
- ¹² Lewis, O. J., *Nature*, **230**, 577 (1971).
- ¹³ Deperet, C., *Arch. Mus. Hist. Nat. Lyon*, **4**, 45 (1887).
- ¹⁴ Oxnard, C. E., *Amer. J. Phys. Anthropol.*, **26**, 219 (1967).
- ¹⁵ Day, M. H., *Nature*, **215**, 323 (1967).
- ¹⁶ Seal, H., *Multivariate Statistical Analysis for Biologists* (Methuen, London, 1964).
- ¹⁷ Tatsuoaka, M. M., *Multivariate Analysis* (Wiley, New York, 1971).
- ¹⁸ Clark, W. E. Le Gros, and Thomas, D. P., *Fossil Mammals of Africa*, No. 3 (British Museum (Natural History), London, 1951).
- ¹⁹ Lewis, O. J., *Amer. J. Phys. Anthropol.*, **36**, 45 (1972).

Positive Allometry of Antlers in the "Irish Elk", *Megaloceros giganteus*

Megaloceros giganteus, the famous Eurasian giant deer of Late Pleistocene times, possessed the largest antlers ever known: reliable estimates of their total span range up to 4 m.

An allometric hypothesis has long been maintained¹ to circumvent the earlier conclusion² that a non-Darwinian orthogenesis had sealed the fate of *Megaloceros* under the oppressive weight of its own enlarging antlers. Simpson³, for example, has argued that selection for an advantageous increase in body size entailed relatively larger antlers as a correlated response—producing a phenotype which, as a whole, proved superior to smaller bodies with relatively smaller antlers. Though this interpretation has the status of textbook dogma^{4,5}, it is based on no biometrical data whatever.

The allometric hypothesis requires two very different tests. (1) Did *Megaloceros*, at its maximal body size for deer of the subfamily Cervinae, lie on a line fitted for adults of all cervine deer? (2) Was the age-standardized variability among adult stags of *Megaloceros* so arranged that selection for larger body sizes entailed relatively larger antlers? Interspecific and intraspecific allometry are quite distinct phenomena^{6,7}.

(1) Data for maximal antler length plotted against shoulder height for living cervines and *Megaloceros* show strong positive allometry⁸ in a reduced major axis fit for error in both variates:

$$\text{Antler length} = 0.0342 (\text{shoulder height})^{1.85}$$

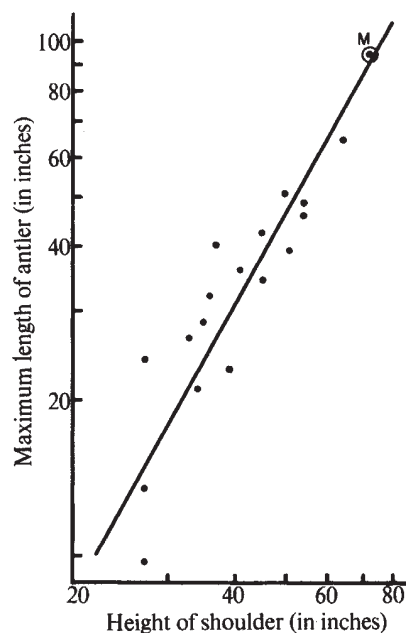


Fig. 1 Allometry among adult cervine stags; each point represents a single species. *Megaloceros* is the circled point at maximum size.

As shown in Fig. 1, *Megaloceros* lies right on the curve and, therefore, represents an expected antler size for deer of its body size. True elk (including the American moose) have relatively small antlers for their body size; giant deer display the expected antler dimensions. (Moose, as members of the subfamily Alcinæ, are not included in Fig. 1. The relatively small antlers of moose compared with those of giant deer may reflect the contrasting habitats of dense thicket and open ground.)

(2) To test the more important second hypothesis, I measured seventy-nine skulls and attached antlers of *Megaloceros* from museums and homes in Eire, Great Britain, continental Europe and the United States. All specimens were found in Ireland or the Isle of Man. In Ireland, *Megaloceros* is known only from sediments of the Alleröd interstadial, a brief period lasting only 1,000 yr⁹⁻¹¹ (G. F. Mitchell (personal communication) doubts the assignment of Savage¹¹ and asserts that no postglacial *Megaloceros* have been found in Ireland). Thus, potential geographical and phyletic components of variation are reduced to a practical minimum for vertebrate palaeontology; one is dealing primarily with true intra-population variation. For antler size, I used a compounded measure of antler length, antler width and lengths of the brow and second tine (the three characters were weighted equally (combining the two tines as a single character) by equalizing the means, applying correlations to each value, summing for each specimen and dividing by three). Because complete skeletons are uncommon, I used basal length of the skull (ventral lip of foramen magnum to anterior tip of premaxillaries) for my measure of body size. This measure has several desirable features: (i) it rapidly reaches a final adult size and remains constant throughout life^{12,13}; (ii) it has a very strong relationship to body length with slight positive allometry¹⁴ (this positive allometry is a welcome feature for a conservative hypothesis; if antlers are positively allometric to the skull, then they will increase with even stronger positive allometry to total body length); (iii) it is the standard dimension of body size in most allometric literature on vertebrates^{6,7}. Fig. 2 shows a grouped plot (at 10 mm intervals of skull length for adult stags); positive allometry is very strong and the log correlation coefficient is 0.987:

$$\text{Antler size} = 0.0000437 (\text{basal length})^{2.584}$$

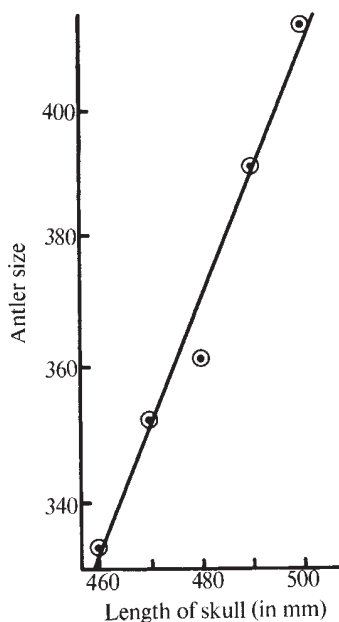


Fig. 2 Intraspecific allometry among adult stags of *Megaloceros*. Data are grouped at 10 mm intervals of skull length; scales are logarithmic.

(The log correlation coefficient for raw data is a highly significant 0.568. An attempt to remove the effect of age upon the antlers by correcting according to the most reliable criterion of pedicel height yielded no significant difference either in the correlation or the parameters of the regression.) The bony pedicel from which the antlers spring decreases in height with the addition of each new pair of antlers^{15,16}. (By plotting the parabolic relationships of antler size against pedicel height (both young and old stags have smaller antlers than animals in their prime), a criterion for the removal of age effects can be constructed.)

The allometric relation is a fact; but the standard interpretation—that the enormous antlers are a passive consequence of advantageously larger bodies—need not be maintained. The relation caters just as well to the idea that selection operated on the antlers or on both antlers and body. Traditionally, the antlers have posed a dilemma because all authors upheld the (often hidden) assumption

that they must function as weapons for battle against predators or rival males (they are poorly designed and over massive for such a role). Field studies of cervids and bovids¹⁷⁻²⁰ have, however, shown that antlers often function primarily as “visual dominance-rank symbols”²¹. As devices for display rather than combat, relatively larger antlers would have an immediate and potent adaptive significance: they would have assured access to females and success in reproduction. Moreover, the antlers of *Megaloceros* are ideally formed for such a role. Deer with palmated antlers show the full palm in prominent display²². To do this, fallow deer and caribou must rotate their heads from side to side (personal communication from V. Geist, December 1972). If *Megaloceros* had to swing its 85 pound antlers about its 5 pound skull, the resultant torque would have posed a mechanical problem. But, uniquely among deer with palmated antlers, *Megaloceros* displayed its full palm when it simply looked straight ahead (Fig. 3). (Once the display hypothesis is entertained, this point is so obvious that it was noted independently by at least three observers: R. Coope, V. Geist and myself; priority must go to Coope.)

I conclude that *Megaloceros* exploited the positive allometry of its antlers to attain the selective advantages of both larger bodies and relatively larger antlers.

STEPHEN JAY GOULD

Museum of Comparative Zoology,
Harvard University,
Cambridge, Massachusetts 02138

Received March 16; revised April 30, 1973.

- ¹ Huxley, J., *Problems of Relative Growth* (MacVeagh, London, 1932).
- ² Lull, R. S., *Organic Evolution* (Macmillan, New York, 1924).
- ³ Simpson, G. G., *The Major Features of Evolution*, 286 (Columbia University Press, New York, 1953).
- ⁴ Dott, R. H., and Batten, R. L., *Evolution of the Earth*, 87 (McGraw-Hill, New York, 1971).
- ⁵ Raup, D. M., and Stanley, S. M., *Principles of Paleontology*, 272 (W. H. Freeman, San Francisco, 1971).
- ⁶ Gould, S. J., *Biol. Rev.*, **41**, 587 (1966).
- ⁷ Röhrs, M., *Z. Wiss. Zool.*, **162**, 1 (1959).
- ⁸ Data from Dollman, J. G., and Burlace, J. B., *Rowland Ward's Records of Big Game* (Rowland Ward, London, 1928).
- ⁹ Mitchell, G. F., and Parks, H. M., *Proc. Roy. Irish Acad.*, **52**, 291 (1949).
- ¹⁰ Flint, R. F., *Glacial and Quaternary Geology*, 404 (Wiley, New York, 1971).
- ¹¹ Savage, R. J. G., *Irish Nat. J.*, **11**, 241 (1955).
- ¹² Rautenbach, I. L., *Ann. Transv. Mus.*, **27**, 83 (1971).
- ¹³ Gottschlich, H. J., *Beitr. Jagd Wildforsch.*, **3**, 81 (1963).
- ¹⁴ Gottschlich, H. J., *Beitr. Jagd Wildforsch.*, **4**, 83 (1965).
- ¹⁵ Reynolds, S. H., *A Monograph of the British Pleistocene Mammalia; The Giant Deer* (Palaeontographical Society, London, 1929).
- ¹⁶ Goss, R. J., *Clin. Orthoped.*, **69**, 227 (1970).
- ¹⁷ Beninde, J., *Monog. Wildsäugetiere*, 4 (P. Schöps, Leipzig, 1937).
- ¹⁸ Darling, F. F., *A Herd of Red Deer* (Doubleday, New York, 1964).
- ¹⁹ Geist, V., *Mountain Sheep* (University of Chicago Press, 1971).
- ²⁰ Kelsall, J. P., *The Migratory Barren-ground Caribou of Canada* (Dept. of Indian Affairs and Northern Dept. of Canadian Wildlife Service, 1968).
- ²¹ Geist, V., *Evolution*, **20**, 566 (1966).
- ²² Geist, V., *Quat. Res.*, **1**, 292 (1971).
- ²³ Hart, J., *A Description of the Skeleton of the Fossil Deer of Ireland, Cervus megaloceros* (R. Graisberry, Dublin, 1830).

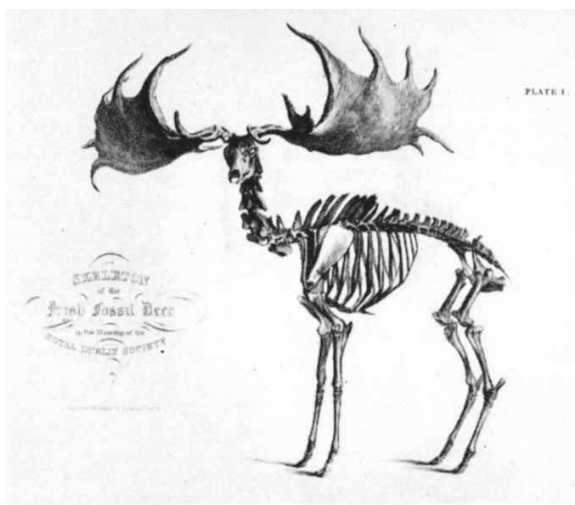


Fig. 3 *Megaloceros* showing the full palm display when looking directly forward. This figure, published in 1830 (ref. 23), represents the second complete (and first reasonably accurate) mounted skeleton. It is still displayed at the National Museum of Ireland in Dublin.

Reference Abbreviations

FROM September 1 it is *Nature's* intention that all abbreviations of references should conform to the style of the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). The changeover will be gradual and authors submitting manuscripts from now on are asked to ensure that the references are written appropriately.

Obituary

T. G. B. Osborn

THEODORE GEORGE BENTLEY OSBORN, Professor Emeritus of Oxford and Adelaide Universities, was born on October 2, 1887. He was educated at Burnley Grammar School and the University of Manchester. His early research was on the fungi and was done mainly during the period 1908–1912, when he was Lecturer in Economic Botany in the University of Manchester. In 1912 he was invited to become Professor of Botany in the University of Adelaide and Consulting Botanist to the Government of South Australia. Shortly before going to Australia, he had married Edith May Kershaw, who was also a botanist and associated with the work of the Botany Department in Adelaide. They had three sons, Peter, Andrew and Richard, all of whom served in World War II; Andrew, an RAF pilot, was killed in action.

When he established the Department of Botany at Adelaide in 1912 Osborn was only twenty-five years old and youthful in appearance. He was fond of the story of how he went into the examination hall, which was being supervised by a middle-aged woman who had not met him, and asked for the Botany I paper. She brought him the paper, and when he said, "And while I'm here I'll have a look at the Agricultural Botany paper, too," she replied, "You'll do nothing of the sort; you'll just sit down there and get on with your Botany I".

Osborn recognized the need for establishing plant ecology in Australia, and began the work on the ecology of South Australia for which he was well known. He occasionally undertook other work such as the descriptions of the morphology of *Isoetes* and *Phylloglossum*. In collaboration with Professor R. S. Adamson (University of Capetown) he published on the ecology of the Ooldea district in 1922 and on the ecology of the Mount Lofty Ranges in 1924. One of his most important contributions was the establishment, in 1924, of the Koonamore Vegetation Reserve, which is now the oldest biological station in Australia with continuous records. The first and important work on grazing of natural vegetation in arid regions resulted from the collaborative work of Osborn, Wood and Paltridge in this area.

In 1928, Osborn became Professor of Botany at the University of Sydney

and he set about reorganizing the undergraduate courses there. He himself took the first year lectures and plant physiology, as well as ecology. At first he continued to clear up his research arising from the work in arid zones but increasingly he became interested in the vegetation of coastal New South Wales. In 1930 he gave the Livingstone Lectures entitled *Plant Life in the Sydney District*, with an ecological approach. He was interested in the xerophytic properties of the plants characteristic of this relatively high rainfall area. In 1932 his Presidential Address to the Linnean Society of New South Wales was on *The Plant in Relation to Water*, with special reference to the properties of xerophytes. Later Osborn's interest in ecology was associated with starting others on ecological descriptions of parts of New South Wales. In that period he published a paper jointly with Robertson on the ecology of the Myall Lakes vegetation.

Osborn became Sherardian Professor of Botany and a Fellow of Magdalen College, Oxford, in 1937. The Oxford Department was very poorly housed in cramped surroundings in an old building in the Oxford Botanic Garden. The Botanic Garden at Oxford, founded in 1621, had become somewhat neglected but Osborn stimulated collaborative work with the garden, and this continued strongly even after the department, due to Osborn's initiative, moved to the new laboratories. As *Nature* said at the time, "the great improvements in the gardens, which have occurred in recent years, owe much to his expert supervision and advice". The achievement of causing a new laboratory to be built was an important contribution, particularly in the difficult period for planning and constructing buildings in the war and immediate post war years. At the same time Osborn spent much time on university business, and was in demand for advisory work for the Agricultural Research Council, of which he was a member (1945–49), and other public bodies.

In 1953 Osborn retired from the Chair at Oxford and returned to Australia. After a short period of residence in Melbourne he returned to Adelaide to live and became Deputy Master of St Mark's College, of which he had been one of the founders. He gave some courses in the Adelaide Botany Department and

regularly attended the departmental seminars. Many younger members of staff and students appreciated conversation with this veteran of three different universities; he had the distinction of being Professor Emeritus of both Oxford and Adelaide Universities. In 1960 Osborn married Marjorie Sabine and they both contributed to the social life of the Adelaide Botany Department.

Osborn was a good lecturer and teacher who succeeded in attracting students of quality; they valued the association with a man who attempted to give them an understanding of high standards of science. Former Australian students include J. G. Wood (formerly Professor of Botany, Adelaide), N. A. Burges (Vice-Chancellor, University of Northern Ireland), M. R. Jacobs (formerly Director General of Forestry), D. Martin (Officer in Charge, Tasmanian Regional Laboratory, CSIRO), H. K. C. Mair (Director and Chief Botanist, National Herbarium, Sydney), Sir Rutherford Robertson (Head, Research School of Biological Sciences, Australian National University), Lilian Fraser (Chief Biologist, Division of Science Services, NSW Department of Agriculture), Joyce Vickery (formerly Botanist, NSW Department of Agriculture), N. C. W. Beadle (Professor of Botany, University of New England) and Gwenda Davis (Associate Professor of Botany, University of New England).

Announcements

International Meetings

August 27–September 1, **Eighth Leucocyte Culture Conference**. (Professor Kerstin Lindahl-Kiessling, University of Uppsala, The Institute for Medical Genetics, V. Agatan 24, S-75220 Uppsala, Sweden.)

August 31–September 1, **The European Symposium of the International Association of Forensic Toxicologists**. (Faculty of Pharmaceutical Sciences, Dept. of Toxicology, State University of Gent, Hospitaalstraat 13, 9000 Gent, Belgium.)

September 2–7, **First International Congress for Bacteriology**. (Secretariat, 'Kenses', P.O. Box 16271, Tel Aviv, Israel.)

September 2–7, **International Association of Microbiological Societies**. (The Inter-

national Congress for Bacteriology, P.O. Box 16271, Tel Aviv, Israel.)

September 2-7, **First International Congress on Mercury.** (Primer Congreso Internacional del Mercurio, Instituto Tecnológico Metalúrgico 'Emilio Jimeno', Facultad de Ciencias, Universidad de Barcelona, Barcelona 14, Spain.)

September 2-8, **International Union of Food Science and Technology.** (Mr G. D. Brown, The Bord Rink Research Centre, Lincoln Road, High Wycombe, Bucks.)

September 3-6, **International Symposium on Liquid Scintillation Counting.** (Mr M. A. Crook, The Society for Analytical Chemistry, 9-10 Savile Row, London W1X 1AF.)

September 3-6, **Second International Symposium on Nuclear Quadrupole Resonance Spectroscopy.** (Dr Arturo Colligiani, NQR Symposium, Laboratorio di Chimica Quantistica, Via Risorgimento 35, I-56100 Pisa, Italy.)

September 3-7, **Third International Conference on Molecular Sieves.** (Professor W. M. Meier, Institut für Kristallographie der ETH, Sonneggstrasse 5, 8006 Zurich, Switzerland.)

September 3-7, **Isotopes and Radiation Techniques in Studies of Soil Physics and Drainage in Relation to Crop Production.** (Mr Y. Barrada of the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, International Atomic Energy Agency, PO Box 590, Karntnerring 11, A-1011 Vienna, Austria.)

September 3-7, **Third International Conference on Chemical Thermodynamics and a Symposium on Physicochemical Techniques at High Temperatures.** (Professor F. Kohler, Institute of Physical Chemistry,

University of Vienna, Währinger Strasse 42, A-1011 Vienna, Austria (Conference); Professor C. B. Alcock, Dept of Metallurgy and Materials Science, University of Toronto, Toronto 5, Canada (Symposium).

September 4-6, **The Phytochemical Society.** (Dr J. G. Vaughan, Biology Dept., Queen Elizabeth College, Campden Hill Road, London W8, or Professor C. F. Van Sumere, Laboratorium voor Plantenbiochemie, Rijksuniversiteit Gent, 35 K.L. Ledeganckstraat, 9000 Gent, Belgium.)

September 5, **Particle Size Analysis in the Sub-Micrometre Range.** (The Society for Analytical Chemistry, Analytical Division, Chemical Society, 9-10 Savile Row, London Q1X 1AF.)

September 5-7, **International Symposium on the Biology of the Male Gamete.** (The Executive Secretary, Linnean Society, Burlington House, Piccadilly, London W1.)

September 5-7, **Nuclear Structure: Heavy Ions and Related Topics.** (Nuclear Physics Sub-Committee, Institute of Physics, 47 Belgrave Square, London SW1X 8QX.)

September 5-7, **Magnetic Bubbles.** (Dr E. A. D. White, Electrical Engineering Dept., Imperial College, Exhibition Road, London SW7.)

September 5-12, **Second International Congress of Plant Pathology.** (Dr Roy D. Wilcoxson, The American Phytopathological Society, 1821 University Ave., St Paul, Minnesota 55104, USA.)

September 5-15, **Large Scale Applications of Superconductivity and Magnetism.** (Drs Simon Foner and Brian B. Schwartz, Francis Bitter, National Magnet Lab.,

Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA).

September 7-9, **More Learning—Less Teaching.** (Meeting Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX.)

September 7-11, **Economic and Social Values in the Assessment of Crop Protection and Pest Control Methods.** (Mr D. T. Sagers, Chesterford Park Research Station, Saffron Walden, Essex.)

September 8-14, **First International Congress of Ecology.** (International Congress of Ecology, c/o Royal Netherlands Academy of Sciences and Letters, Kloveniersburgwal 29, Amsterdam, The Netherlands.)

September 8-15, **Eighth International Congress of Chemotherapy.** (Scientific Secretary, Dr Polyxeni Kntomichalou, PO Box 1554, Athens, Greece.)

September 9-14, **Third International Congress of the International Radiation Protection Association.** (Mr John C. Villforth, Secretary, Bureau of Radiological Health, FDA, 5600 Fishers Lane, Rockville, Maryland 20852, USA.)

September 10-11, **Third Biennial Symposium on Turbulence in Liquids.** (Dr J. L. Zakin, Dept. of Chemical Engineering, University of Missouri-Rolla, Rolla, Missouri 65401, USA.)

September 10-11, **Solid State Devices Conference.** (The Solid State Physics Sub-Committee of the Institute of Physics, 47 Belgrave Square, London SW1X 8QX.)

September 10-12, **Carbonization and Graphitization.** (Conference Secretary, Dr H. Marsh, Northern Coke Research Labs., School of Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU.)

HOW TO BUY NATURE

The cost of one year's subscription to NATURE is:

	UK & elsewhere	US & Canada
Nature (Friday)	£16	£20
Nature & Nature Physical Science or Nature New Biology	£24	£33
Nature New Biology or Nature Physical Science	£10	£15
All three editions	£29.50	£44
Index (1972)	£1	£1.50

(Charges for delivery by air mail on application). Subscribers in North America may be able to claim a tax rebate against their NATURE subscription.

Editorial, Advertising and Publishing Offices of NATURE

MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number: 01-836 6633. Telegrams: Phisus London WC2R 3LF
Telex 262024

MACMILLAN JOURNALS LIMITED
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number: 202-737 2355. Telex 64280

Advertisement Department
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Numbers: UK 01-836 6633. USA 202-737 2355

Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS RG21 2XS
Telephone Number: Basingstoke 29242

Classified advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone Number: 01-242 6264/01-405 4743
Telegrams: Textualist London WC2A 2ED
Registered as a newspaper at the Post Office